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ON A NEW DEEP-WATER TRACHYMEDUSA

By F. S. Russell, D.Sc., F.R.S.
The Plymouth Laboratory

(Text-fig. 1)

In August and September 1959 a series of deep horizontal hauls with a 2 m stramin closing net were made on R.R.S. 'Discovery II' for Dr T. H. Waterman to study the vertical distribution of the larger plankton animals. The collections were made within the two areas 32° 22′–32° 49′ N., 21° 14′–22° 16′ W. and 37° 28′–37° 56′ N., 11° 59′–13° 46′ W.

I have examined the medusae in these collections, and among them were eighty specimens of a trachymedusa which did not agree with any previously described species. I sent some specimens to Dr P. L. Kramp and he also was unable to identify them.

The medusa is *Aglantha*-like in shape and general appearance, but as its description will show it can be placed in the genus *Colobonema*. On account of its shape I am naming it *Colobonema apicatum* n.sp.

Most of the specimens were in a fair state of preservation. The umbrella is tall and cylindrical with a small apical process. The jelly is fairly thick; along the sides of the umbrella it is slightly thicker in the upper region becoming thinner as the margin is approached. The subumbrellar musculature is well developed. In the apical region of the umbrella (Fig. 1 B) the flat circular area is devoid of musculature, as in *Pantachogon haeckeli*. In this respect it differs from *Colobonema sericeum* (see Russell, 1953, pp. 437, 441). Through this muscle-free area the eight radial canals can be seen running downwards along a very short conical peduncle to the centre of the stomach. The stomach, which is also very short, has four simple perradial lips, and seen from above its wall appears to be divided longitudinally into eight lobes (Fig. 1 B). The ring canal and the distal portions of the radial canals are filled with fat globules.

The gonads cover the subumbrellar walls of the radial canals for about two-thirds of their length. No specimens still retain all eight gonads complete, but there are usually short pieces of gonad left attached at the proximal and distal ends (Fig. 1 A). Sometimes a terminal portion is hanging loose and might be mistaken for a pouch.

The marginal tentacles all appear short and stump-like. They are probably broken off without their ectodermal covering, as they usually are in *C. sericeum*. In the latter species it was uncertain whether the short stumps represented the whole tentacle (Russell, 1953, p. 438), but I have now seen

specimens of that species with long thread-like tentacles which are very delicate and easily broken off.

In no specimen have I seen a complete number of marginal sense organs, but sufficient are present to show that typically there are sixteen, each separated by a pair of tentacles. They are typical free sensory clubs and each has a single concretion.

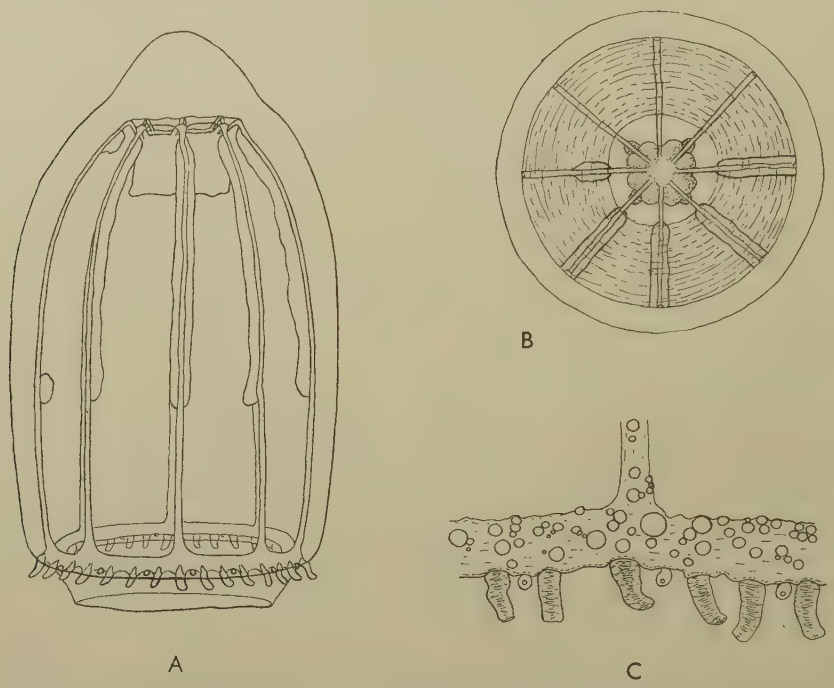

Fig. 1. *Colobonema apicatum* n.sp. A, Type specimen, 10 mm high; B, apical view of umbrella of type specimen showing area devoid of musculature; C, exumbrella view of umbrella margin of another specimen to show arrangement of marginal sense organs in relation to tentacles, and fat globules in canals; the velum is omitted.

I have chosen one of the largest and best preserved specimens as the type. This has been deposited in the British Museum and has the catalogue number B.M. 1960.11.20.1. This specimen is shown in Fig. 1 A, B. The umbrella is 10 mm high, and 6·16 mm wide at its widest point and 5·83 mm wide at the umbrella margin. The height of the apical process is 1·7 mm. The jelly of the umbrella is 0·66 mm thick in the upper portion and 0·33 mm at the umbrella margin. The velum is 1·33 mm wide. The stomach, with peduncle, is 1·33 mm long. The gonads are 5·33 mm long; only four are intact. The marginal tentacles are 0·33 mm long.

The diagnosis of the species can now be given as follows, based on measurements of a number of specimens.

Colobonema apicatum n.sp.

Umbrella deep cylindrical bell-shaped, with small conical apical process; width of bell somewhat more than half its height, velum broad and well developed. Stomach small, situated on very short peduncle, its length about one-sixth of umbrella height. Mouth with four simple lips. Eight straight narrow radial canals and ring canal. Eight narrow linear gonads, without folds, along almost the proximal two-thirds of radial canals. Thirty-two solid marginal tentacles. Sixteen marginal sense organs. Umbrella up to 10 mm in height. Whole medusa colourless, subumbrellar musculature iridescent.

VERTICAL DISTRIBUTION

An analysis of the vertical distribution of the species for those stations at which the medusae occurred is given in Table 1. It is evident that *C. apicatum* normally lives very deep in the water and that the majority occur below 1500 m. No differences in vertical distribution could be detected between day and night but the numbers of specimens were rather low to be conclusive.

TABLE 1

Depth (m)	No. of hauls	Total no. of specimens	Specimens/ hauls
650	2	—	—
830– 890	4	—	—
970–1050	7	—	—
1100	1	—	—
1200–1290	6	5	0·8
1370–1450	8	7	0·9
1600–1690	6	33	5·5
1770–1850	6	35	5·8

SUMMARY

A new species of deep-water trachymedusa from the North Atlantic is described and named *Colobonema apicatum*. Most specimens occurred deeper than 1500 m.

REFERENCE

Russell, F. S., 1953. *The Medusae of the British Isles*. Cambridge University Press.

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ON THE NUTRITION AND METABOLISM OF ZOOPLANKTON

I. PRELIMINARY OBSERVATIONS ON THE FEEDING OF THE MARINE COPEPOD, *CALANUS HELGOLANDICUS* (CLAUS)

By E. D. S. Corner
The Plymouth Laboratory

Although much is already known about the diets of certain marine animals and the culturing of various unicellular algae, there is still a good deal to be learned about the quantitative aspects of energy transfer in oceanic food chains. One aspect of this problem which has attracted considerable attention is the grazing of the phytoplankton by certain species of zooplankton, and particular interest has been shown in the feeding habits of the copepod *Calanus finmarchicus* (Gunnerus). Studies made with this animal have been concerned with determining various species of diatom or flagellate on which it will feed in the laboratory; also with the proportion of ingested food it actually assimilates; and, especially, with attempts to estimate the rate at which it filters food from the surrounding medium (Clarke & Gellis, 1935; Fuller & Clarke, 1936; Fuller, 1937; Harvey, 1937; Raymont & Gross, 1942; Gauld, 1951; Marshall & Orr, 1955*b*, 1956; Conover, Marshall & Orr, 1959).

Results, in general, show that *C. finmarchicus* grazes at markedly different rates, depending on the diet used; in addition, there is even considerable variation between different estimates of the grazing rate found with one particular species of diatom (e.g. *Ditylum brightwellii*) or flagellate (e.g. *Chlamydomonas*); and more recent work by Conover *et al.* (1959) has shown that these values can vary greatly depending on the method of estimation. For example, a grazing rate determined by the technique of counting cells can be as high as 224·7 ml./animal/day, whereas that found using ^{32}P-labelled diatoms can be as low as 11·5.

In order to estimate the amount of food assimilated by *Calanus* it is necessary to take into account several factors other than the filtering rate. For instance, it is equally important to know the quantity of the diet; and the concentration of the food in the surrounding medium. Moreover, in order to assess the adequacy of any filtering rate for sustaining the animal in its natural habitat it is necessary to know the concentration and chemical composition of particulate matter in the sea. Unfortunately, this kind of information has not been obtained by previous workers and it is therefore difficult, without making several lengthy extrapolations, to elucidate the full meaning of their results.

In addition, laboratory experiments with *Calanus* have frequently been carried out in a closed system, the animals being confined in a fairly small volume of sea water enriched with a culture of a single species of phytoplanktonic organism, and always in contact with their excretory products.

These various considerations have been borne in mind during the present study, and the results obtained, unlike those of previous workers, are in complete accord with the view that *Calanus* satisfies its food requirements by filtering particulate material from the surrounding sea water. There is no evidence that dissolved organic substances make a substantial contribution to its diet.

METHODS

Animals. Calanus helgolandicus (Claus) (see Rees, 1949; Russell, 1951) was collected with a coarse tow-net (25 m.p.i.), of 1 m diameter, from the surface of the sea in the area between Penlee Point and Renny Rocks, at the entrance to Plymouth Sound. As female *Calanus* eat more than males (Raymont & Gross, 1942) only females were used in the present work; and their separation from the tow-nettings was made easier by the fact that they greatly preponderated, possibly because the hauls were made near the surface (see Russell, 1928). Quite a few of the animals bore spermatophores and others were at the so-called ripe stage, although there was little evidence of egg-laying during the experiments. Once the animals had been sorted they were immersed overnight in sea water containing a mixture of penicillin and streptomycin (25 mg. of each/l.), then washed with fresh sea water and transferred to the apparatus.

Continuous flow assembly. This consisted of two series of six glass vessels, each of 200 ml. capacity, arranged in cascade. A sample of sea water (100 l.), collected from the same sea area as the *Calanus*, was passed through a medium bolting silk (48 m.p.i.) to remove macroplankton, then pumped into two large polythene reservoirs, each holding 40 l. The remaining 20 l. of sea water was used for an estimation of the quantity of particulate matter in the sample. To start the experiment, sea water was run from the reservoirs into the glass vessels and an appropriate number of animals was then added to each. In most experiments, one series of vessels contained 96 animals (16/vessel) and the other series 48 (8/vessel). Sea water was then passed continuously through both series of vessels at a steady rate for 4 days. The inlet at the base of each vessel was covered with a glass bead to prevent the animals from moving down into the feed tube: the outlet at the top was similarly protected so that the animals were not swept out of the vessel in the stream of sea water. As the sea water left the last vessel on each side of the assembly it was filtered through a fine bolting silk (180 m.p.i.) to trap any faeces that had inadvertently been removed from the apparatus. At intervals of 24 h the two batches of sea water, one of which had flowed over 96 *Calanus* and the

other over 48, were separately examined for their contents of particulate material, the difference between the two values being a measure of the quantity filtered off by 48 *Calanus*. This procedure obviated the need to take into account any suspended material lost on the sides of the glass vessels or on the walls of the rubber tubing during the passage of the sea water through the apparatus. Nevertheless, had there been a substantial growth of phytoplankton during the 4-day period, it might have seriously affected the results, and so to avoid this the experiments were carried out at 10° C in total darkness. It is doubtful that either of these factors were adverse to *Calanus*; indeed, Fuller (1937) has found that they feed better at 8° C than at a higher (13° C) or lower (3 °C) temperature, and both he, and Marshall (1924), have reported evidence consistent with the view that *Calanus* feeds actively in the dark.

Rate of flow of sea water. As the total volume of each series of vessels was 1·26 l., each of the 96 *Calanus* placed in one side of the apparatus occupied only a little over 12 ml. of sea water. However, because 10 l. of fresh sea water was passed through each series of vessels each day the actual volume of sea water available to each animal was more than 100 ml./*Calanus*/day. Experiments showed that if this volume was markedly reduced many animals did not survive the experiment. On the other hand, if the volume was greatly increased the separation of suspended material to be used in analysis was impracticably long.

The time for which the experiment was continued (4 days) was also dictated by expedience. Thus, if a much longer experimental period was used, the system became contaminated with bacteria; and if the time was greatly shortened, the quantity of food removed was too small for accurate measure.

Usually, more than 90% of the test animals survived the experiment and it might be pointed out that previous workers have recorded that *Calanus* remain healthy for several days (Clarke & Gellis, 1935), or even several weeks (Marshall & Orr, 1955*a*) in fresh sea water to which no additions have been made. However, on one or two occasions in the present work, when the concentration of suspended material in the sea water was low ($<$ 2 mg. dry wt./l.), 20% or more of the animals either died, or turned opaque and moved sluggishly at the bottom of each vessel, a sure sign of distress. When this happened, the experiment was abandoned.

Collection of faeces. It has been found (Marshall & Orr, 1955*b*) that the size of the faecal pellets shed by *Calanus* varies with the diet. Thus, those from females feeding on *Dunaniella* are about 500 μ in length, whereas the corresponding value for animals feeding on *Skeletonema* is 950 μ. In the present work, at the end of each experiment, the animals were pipetted out of the glass vessels, from which the sea water was then strained through a suitably fine bolting silk (180 m.p.i.). The faeces so collected were then added to those retained by the traps below the final vessels in each series of the assembly (see earlier), and the combined sample was then dried and weighed.

Uptake of food. The sea water that flowed each day through each series of glass vessels was filtered under pressure through Oxoid membranes (pore size, 0·5–1·0 μ; membrane diameter, 6 cm), from which, after rapid washing with distilled water, the retained material was removed by scraping with a rubber 'policeman'. After the 4-day experimental period, all the material so obtained was dried, first at 95° C for 30 min, and then at 65° C *in vacuo*, to constant weight (see Collyer & Fogg, 1955). The 20 l. sample of sea water which had not contained any test animals was similarly treated. Thus, three separate amounts of material were eventually obtained, the first being that left in sea water occupied for 4 days by 96 animals (*a*), the second being that remaining in sea water occupied for the same time by 48 animals (*b*), and the third being that present in 20 l. control sea water (*c*). From these values the amount of suspended material removed by the animals was calculated as $(b-a)/(4 \times 48)$ mg/*Calanus*/day.

Analysis of food. The amount of inorganic material in the available food was estimated by ashing; organic nitrogen—and hence protein—by the Kjeldahl method (see Barnes, 1959); lipid by the extraction procedure described by Milner (1948); and carbohydrate by difference.

Selective feeding. Harvey (1937) has shown that *Calanus* have a preference for certain kinds of diet. Therefore, in order to assess the nutritional value of any food, not only was it necessary to analyse its content of carbohydrate, lipid and protein; it was also important to know whether the animals used their feeding mechanism to select certain fractions (e.g. diatoms, flagellates) and reject others (e.g. particulate inorganic material). To examine this latter possibility, comparison was made of the proportion of inorganic material in the particulate matter before and after the animals had fed on it, the assumption being that any change in this quantity would reflect the degree of selectivity practised by the animals during feeding. Unfortunately, it was not possible to measure this accurately after each individual experiment because the animals removed only a small amount of the food available; the estimation had therefore to be based on the combined results of several experiments, and the following example illustrates a typical calculation using data from the last four. Thus, during these experiments the total quantity of suspended material available to the animals was 505 mg (dry wt.) of which 224 mg was in the inorganic fraction and 281 mg in the organic. However, after the animals had fed on this material the total amount left was 461 mg, of which 215 mg was in the inorganic fraction and 246 mg in the organic. Thus, of the 44 mg removed by the animals, 35 mg was organic material and only 9 mg inorganic. Obviously, therefore, the animals must have preferentially selected the organic fraction of their food, for whereas this fraction only accounted for 55·8% of the dry weight of suspended material available, it accounted for nearly 80% of the material actually removed.

Filtering rate. This was estimated as $20{,}000/4 \times 48\,[(b-a)/c](x/y)$ ml./animal/day, where a, b and c are as defined earlier (see 'Uptake of food'), x is the percentage of organic material in the food actually removed by the animals, and y is the percentage of organic material in the food available.

Assimilation of food. The amount of food assimilated by the animals and subsequently used in their metabolism was estimated by subtracting the quantity excreted as faecal pellets from that which had originally been ingested. The quantities of faeces obtained were much too small to be used for analysis, and all calculations are therefore based on the assumption that the faeces had the same chemical composition as the diet. This is probably not true, for the faeces may have contained a considerably higher proportion of unwanted inorganic material, which means that the amount of organic material assimilated may be even higher than that reported, and the respiration rate, as calculated from this, even greater than that directly measured by previous workers. No account was taken of metabolically active substances that may have been lost in the urine or across the body surfaces.

Calculation of oxygen consumption. This was based on the volume of oxygen required to metabolize lipid (1 g $\equiv$ 2·03 l. O_2) and carbohydrate (1 g $\equiv$ 0·75 l. O_2) present in the total quantity of food assimilated. Protein was not considered in the calculation because in normal circumstances it is not used primarily as a fuel.

Accuracy of the continuous flow method. All the estimations of food uptake depended on comparisons of the amounts of suspended material left in the sea water emerging from the two sides of the assembly. Moreover, separation of this material from the sea water and its subsequent estimation involved several procedures which might have introduced experimental errors. To check this, 40 l. of the same sample of sea water was passed through each series of vessels, to which no animals had been added, at a rate of 10 l./day. At the end of 4 days, each of the two emergent samples of sea water was analysed for its content of suspended material, using the methods described earlier. It was found that the total quantity of suspended material collected from one batch of sea water was 124·6 and that from the other 126·2 mg dry wt., a duplicate result of encouraging accuracy, in that the difference between these two quantities during experiments with animals was normally 10–15 mg dry wt.

RESULTS

The results of all experiments made between May and September 1960 are shown in Table 1. Throughout this period of the year the sea water was found to contain 0·95–2·50 mg insoluble organic material/l., values in fair agreement with those of 1·15–1·77 mg/l. reported by Armstrong & Atkins (1950), but less than that of 3·2 mg/l. reported by Jones & Wills (1956). Analyses of this organic fraction showed that carbohydrate accounted for

TABLE 1. THE FEEDING OF *CALANUS HELGOLANDICUS* IN A CONTINUOUS FLOW OF 'OUTSIDE' PLYMOUTH SEA WATER

All experiments conducted in the dark at 10° C. Calculations as described in text.

Expt.	Time of year	Particulate material in sea water (mg dry wt./l.)		Quantity removed daily by *Calanus* (μg dry wt./animal)	Quantity assimilated daily by *Calanus* (μg dry wt./animal)	Daily assimilation of organic material (μg dry wt./animal)			Dry wt. of *Calanus* (μg/animal)	% Dry wt. digested daily	Filtering rate (ml./day/animal)	Oxygen consumption (μl./day/animal). Calculated from amount needed to metabolize digested food		Oxygen consumption (μl./day/animal). Measured for *C. finmarchicus*
		Inorganic	Organic			Carbohydrate	Lipid	Protein				Lipid	Carbohydrate	
1	May	3·45	2·35	34·0	25·8	13·5	5·6	1·7	103	20·2	11·4	10·2	10·1	17·8*
2	May	1·79	0·98	44·5	41·2	20·8	8·8	2·7	—	31·4	36·0	17·9	15·6	—
3	June	2·56	1·77	36·5	31·2	15·6	6·4	1·9	117	20·4	16·3	12·6	11·7	6·7*
4	June	3·64	2·50	31·7	24·2	13·7	3·9	2·2	—	16·9	10·0	7·9	10·3	—
5	July	1·75	0·95	43·0	40·5	22·7	5·6	3·6	102	31·2	35·5	10·2	17·1	12·9*
6	July	1·38	1·72	58·3	43·5	24·4	6·1	3·9	—	33·5	25·7	12·4	18·3	—
7	Aug.	1·48	1·88	26·0	21·0	10·6	4·2	1·7	104	15·9	10·9	8·5	7·9	8·6*
8	Aug.	1·29	1·84	66·5	47·1	23·7	9·9	3·8	—	35·6	28·4	20·2	17·7	—
9	Sept.	1·34	1·68	42·6	33·6	16·3	6·5	2·6	98	25·9	20·1	13·2	12·2	14·4†
10	Sept.	2·76	1·92	51·1	39·1	19·8	7·9	3·2	—	31·5	21·1	16·1	14·8	—

* Data from Marshall, Nicholls & Orr (1935); † Data from Raymont & Gauld (1951). All values temperature-corrected to 10° C.

64·1–70·8%, lipid 17·7–27·1% and protein 8·3–11·5% of the dry weight, average values being 66·5, 23·4 and 10·1 respectively, as compared with corresponding values of 63, 8 and 29 reported by Brandt (1898) for the marine diatom *Chaetoceros* and those of 85, 1·4 and 13·6 reported by Ketchum & Redfield (1949) for the marine dinoflagellate *Ceratium*.

Usually, about 80% of the food taken up by the animals was actually digested and it should be noted that high values for this have also been obtained by Marshall & Orr (1955*b*) when feeding *Calanus* with certain diatoms and dinoflagellates. Moreover, because of their selective feeding, the animals assimilated a diet containing a high percentage of organic material, and the average daily quantities of carbohydrate, lipid and protein which they digested were 18·1, 6·5 and 2·7 μg, respectively. The total amount of organic material assimilated daily, expressed as a fraction of the dry weight of the animals, is also shown in Table 1 and the average value, based on the results of ten experiments, is 25·3%.

It should be noted that the animals used in the present work were much smaller than those studied earlier at Plymouth by Bogorov (1934). Thus, in the present investigation, the dry weight varied between 98 and 117 μg per animal with an average value of 106 μg, whereas Bogorov reported May–September values between 254 and 193 μg with an average of 210 μg. A possible explanation of this discrepancy is that dry weights in the present work were determined after the animals had been dried to constant weight at 100° C, whereas in Bogorov's experiments the animals were dried in a desiccator at room temperature, and then not necessarily to constant weight. It should be pointed out that Marshall, Nicholls & Orr (1934) describe the dry weight of female *Calanus* caught during the period May–September in the Clyde area as varying between 250 and 132 μg; but Marshall & Orr (1955*a*) also report a sample caught in July 1932 with an average dry weight of 113 μg, which closely accords with the values found in the present work. It seems, therefore, that the size and weight of *Calanus* can vary considerably with time and place, doubtless reflecting changes in the temperature or nutrient content of the sea water (see Russell, 1935, 1936, 1939).

DISCUSSION

Pütter (1909) claimed that many marine animals, including copepods, could not obtain sufficient food in the open sea by filtering off suspended material, but must make substantial use of dissolved organic substances which are absorbed through their body surfaces. In support of this hypothesis, Pütter quoted values for the amounts of dissolved organic matter in sea water which later workers (Moore, Edie, Whitley & Dakin, 1912; Krogh, 1931) have shown to be much too high. He is also thought to have overestimated the oxygen requirements of *Calanus* (Pütter, 1922), claiming that at 17·7° C it uses 33·5 μl./day, whereas Marshall, Nicholls & Orr (1935) report a corresponding

value of only 17·8 μl./day. Again, Pütter calculated that *Calanus* requires 38·7% of its dry weight/day as food, whereas according to Marshall *et al.* adult *Calanus*, during the summer, need only between 6·2 and 7·6%.

Nevertheless, although Pütter's hypothesis has long been regarded as suspect, the results of many workers who have studied the feeding of *Calanus* have lent indirect support to his views. Thus, the respiration rate of *Calanus*, as determined by Marshall *et al.* (1935), has often been used in order to calculate the quantity of food that the animals need daily; and the rate of filtering required to accumulate this quantity of food in the open sea has been compared with the corresponding values determined in the laboratory. Usually, the filtering rate, as measured in the laboratory, is much too low. Thus, Fuller & Clarke (1936) estimated that their animals would have to filter sea water at a rate of 72 ml./day in order to accumulate their requirements of food, whereas the maximal rate measured in the laboratory was only 5·61. Likewise, Fuller (1937) found that the rate of filtering under laboratory conditions was only about one-tenth of what was needed to sustain the animals in the open sea. A similar conclusion was reached by Raymont & Gross (1942) and by Marshall & Orr (1955*b*). However, Harvey (1937) and Gauld (1951) reported much higher filtering rates, and Gauld concluded that the copepods used in his experiments could probably have obtained sufficient particulate food in the open sea by filtering a daily volume of sea water at a rate corresponding to that which he measured in the laboratory. Unfortunately, however, he did not provide any data concerning the amount of food in the phytoplankton available to his animals in the sea, and his results did not therefore exclude the possibility, re-stated by Marshall & Orr (1955*a*), that an apparent gap exists between the amount of particulate food present in the sea and the quantity required daily by *Calanus*.

Cushing (1959) draws attention to some results which he thinks may possibly resolve this difficulty. In experiments with the copepod *Temora longicornis* he found that the filtering rate of the animal greatly increased with the volume of sea water in which it was confined. For example, raising this volume from 25 to 100 ml. increased the daily filtering rate from 26 to 150 ml. However, Marshall & Orr (1955*b*) found that although *Calanus* had a very low filtering rate when confined in a small volume of sea water (1 animal/ml.), once this volume was increased beyond a certain level (1 animal/3·3 ml.), there was no further rise in filtering rate. Furthermore, the results of the present work are in agreement with this in that the volume of sea water available to the animals was much larger (1 animal/100 ml.) and yet their filtering rates, which varied between 10·0 and 36·0 ml./animal/day (average 21·5), are all within the range (1–84 ml.) reported by Marshall & Orr.*

* Marshall & Orr (private communication) now consider their values for filtering rates to be underestimated by 10–50%. They also report further work with *Calanus* and *Temora* in which they have been unable to confirm Cushing's findings.

Interpretation of the results obtained in the present work does not require the assistance of Pütter's hypothesis. Indeed, the quantities of lipid and carbohydrate assimilated by the animals were considerably in excess of those needed to account for an oxygen consumption similar to that reported by Marshall *et al.* (1935) or by Raymont & Gauld (1951). Thus, the average amount of lipid digested was equivalent to 12·9 and the average quantity of carbohydrate equivalent to 13·6 μl. O_2/animal/day; whereas the average respiration rate, as determined by previous workers, was only 12·1 μl. O_2/animal/day. It appears, therefore, that the animals digested roughly twice the quantity of food needed to account for their respiration rate, and in order to explain this difference it is necessary to consider the following possibilities.

The first of these is that all the food taken in by the animals may not have been metabolized; some may have been stored in the tissues. To account for the difference between the amount of food digested/day/animal (25·3% of the dry body weight), and the quantity equivalent to the oxygen consumption (which varies from 5·6 to 15·2%, depending on whether all fat or all carbohydrate is metabolized), the animals would have had to increase their dry body weight daily by a value between 10·1 and 19·7. As the number of animals used never exceeded 150, it was not possible to make an accurate estimate of the extent of their growth during any one experiment. However, a single determination, carried out using animals pooled from all ten experiments, indicated that the daily increase in dry body weight was only 2–3% which, far from accounting for the difference between food digested and food metabolized, is barely equivalent to the daily assimilation of protein.

A second possibility is that the animals do not metabolize carbohydrate by a purely oxidative mechanism but rely to a considerable extent on anaerobic glycolysis and excrete the lactic acid so formed. This, however, seems unlikely because Marshall *et al.* (1935) have shown that *Calanus* quickly die in sea water of markedly lowered oxygen content.

A third, and more likely, possibility is that the animals, under the conditions of the present experiments, respired at a rate faster than those described by previous workers; and, concerning this point, it seems relevant to draw attention to the considerable variation in the values so far reported. Thus, for stage V *Calanus* at 17° C, Clarke & Bonnet (1939) describe a value of 22·8 μl. O_2/animal/day, whereas Raymont & Gauld (1951) report a corresponding figure of 12·5, and Marshall *et al.* (1935), for a slightly lower temperature (15° C), quote a value of 10·1. In addition, of course, these results have all been obtained using *C. finmarchicus*, comparatively few observations having been made with *C. helgolandicus*, the species used in the present work. Marshall & Orr (1958), however, have recently made some measurements of the respiration rate of this latter species and, from their results with both ripe and unripe females caught during winter months (no summer values are

quoted), the average value determined for animals at 10° C may be calculated as 8·4 μl. O_2/*Calanus*/day, as compared with a corresponding figure of 7·4 for *C. finmarchicus*. There is therefore evidence that *C. helgolandicus* respires at a slightly faster rate. In addition, it might be expected that animals which are continuously feeding, as in the present study, have a metabolic rate higher than that of animals kept in filtered sea water, as in studies on respiration rates by previous workers: and evidence consistent with this view has been reported by Conover (1956) who showed that the respiration rates of certain copepods are markedly lowered when the animals are starved. Thus, that of *Acartia clausi*, averaged over a 4-day period, falls from 0·92 to 0·71 μl. O_2/animal/day; and that of *A. tonsa* from 1·57 to 1·10. These various considerations stress an obvious need to measure the respiration rate of *Calanus helgolandicus* under conditions similar to those used in the feeding experiments; and it seems premature to discuss the wider implications of the present findings until these measurements are made.

Finally, it should be emphasized that although the results of this work indicate that *Calanus* obtains more than enough food to account for its respiration rate by filtering particulate organic material from the surrounding sea water, this is not to say that Pütter's hypothesis is completely wrong. For even though the animal assimilates the bulk of its diet in this way, it might still have to rely to some extent on traces of dissolved organic substances, absorbed through the body surface, in order to sustain normal, healthy development. Furthermore, all the present experiments were carried out during summer months, and so the question of whether *Calanus* makes considerable use of dissolved organic substances in winter remains undecided.

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SUMMARY

Measurements have been made of the quantity and type of food assimilated by adult female *Calanus helgolandicus* (Claus) during the summer months.

Animals kept in the dark at 10° C under a continuous flow of 'outside' Plymouth sea water (0·95–2·50 mg insoluble organic material/l.) removed 26·0–66·5 μg/day/*Calanus*, of which 74–91 % was actually digested.

Volumes of sea water filtered daily varied between 10·0 and 36·0 ml./animal, with an average of 21·5.

The animals preferentially selected a diet of high organic content from the particulate food available, digesting average daily quantities of 18·1 μg carbohydrate, 6·5 μg lipid and 2·7 μg protein.

The average amount of food digested daily accounted for 25·3% of the dry weight and was equivalent to an average respiration rate of 26·5 μl. O_2/animal/day, which adequately accounts for the highest values reported by others for *C. finmarchicus* (Gunnerus) kept under similar conditions of light and temperature.

These results, which provide direct evidence that *Calanus* obtains the bulk of its food from particulate material present in the sea, have been discussed with especial reference to Pütter's hypothesis.

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THE DISTRIBUTION OF SOME PLANKTON ANIMALS IN THE ENGLISH CHANNEL AND WESTERN APPROACHES

I. SAMPLES TAKEN WITH STRAMIN NETS IN 1955 AND 1957

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(Text-figs. 1–6)

Our knowledge of the distribution of plankton in the English Channel and farther west has never been complete since work at Plymouth does not normally include routine surveys made over a long period. Recently, an opportunity was taken to carry out further discontinuous surveys in the hope that enough evidence could be gained to allow comparison with previous work and allow re-assessment of the long-term changes in distribution that appear to have occurred in the area during the past fifty years.

This first contribution describes the results of samples taken with stramin young fish trawls. The cruises were probably the last occasions that the young fish trawls will be used from Plymouth for other than qualitative sampling; and on later cruises, to be dealt with in another paper, high-speed samplers were used. It has been thought worth while therefore to give a brief outline of previous investigations with conventional nets, before the account of the work in 1955 and 1957.

PREVIOUS PLANKTON RESEARCHES AT PLYMOUTH

The earliest surveys were those carried out by the M.B.A. on behalf of the Government, as part of the International Investigations begun in 1903. The work in the English Channel ended in 1909 when the International programme was transferred to the newly established Government Fisheries Laboratory at Lowestoft. From 1903 to 1909 four cruises were made each year to a gradually increased number of stations at which hauls were taken with horizontal and oblique townets and the vertical quantitative Hensen net (Gough, 1905, 1907; Bygrave, 1911). The results were reported by these authors in a somewhat compressed form on a semi-quantitative basis, but with a detail of species never attempted since. Unfortunately, organisms were classed only as oceanic or neritic and it is difficult to compare the results with later investiga-

tions in which emphasis was given to different types of organisms (e.g. Russell, 1935).

Plankton work started again at Plymouth in 1924 and continued in detail up to 1935. As part of his now classic researches on the local plankton, Russell (1935) revised some earlier observations of Meek (1928) on the distribution of *Sagitta* in relation to hydrography and the herring fishery. The work was expanded to include several macroplankton species occurring off Plymouth, and later in other regions, and their relationship to different water masses demonstrated (Russell, 1935, 1936, 1939). Russell discovered the great change that took place in the macroplankton in Plymouth coastal waters in 1930–31, when many animals (including young fish) disappeared or became rare coincidently with a fall in nutrient salt levels in the water during the winter. The weekly plankton hauls, planned to follow these changes, using the 2 m stramin ring trawl, have been continued at less frequent intervals to the present day, but not all have been analysed or reported upon.

After 1935, further surveys, using the 1 or 2 m stramin ring trawls were made in the Western Approaches to the Channel, as part of an International Investigation of the mackerel fishery (Corbin, 1947). Since the war stramin ring trawl hauls have been taken west of Plymouth on several fishery and hydrographic cruises, but the results have not been presented. There is a growing appreciation that the stramin ring trawl is not a good quantitative sampler, in part owing to the variation in mesh size of manufactured samples of stramin supplied since 1945.

THE STRAMIN RING TRAWL AND METHODS

The method of operating the young fish trawl, to give it its common name, has been described by Russell (1930); the maximum depth reached in a haul varies from 30 to 50 m. There are several advantages compared with ordinary tow-nets (and even the larger types of quantitative Hensen or Helgoland net) in using the ring trawl for studying the distribution of young fish, their eggs, and large plankton animals. It takes a big sample, theoretically 4000 m^3 being filtered in a half-hour oblique haul at an estimated two-thirds efficiency; it collects the larger 'indicator' species, especially the coelenterates, in good condition; it does not capture the large mass of herbivorous copepods which are less useful as 'indicators', but retains some at least of the more useful spiny predatory forms such as *Euchaeta* and *Candacia;* and in theory its filtration efficiency (not yet measured) should be good because of the proportionately long bag (6 m) compared with the aperture of 2 m.

There are several disadvantages apart from the post-war variation in mesh. The most serious defect is that the depth of fishing and the volume of water filtered is critically dependent on the weight of warp and the speed of the ship through the water. Since oblique hauls are made at very low speed (*ca* 2 knots)

the ship is affected by the weather, and is difficult to maintain on course or at a given speed. In the M.B.A.'s latest vessel, 'Sarsia', the main engines cannot be operated below 5 knots; the auxiliary drive of 20 h.p. is therefore employed for ring trawl hauls, and speed through the water, if any, is an unknown variable. Even hauls made by the smaller diesel ships, 'Sula' and 'Sabella', which are (or were) better powered than 'Sarsia', can hardly be compared with Russell's work with the steamboat 'Salpa', on which the warp angle could be kept reasonably constant. Another serious defect, which applies to all slowly hauled nets, is the difference between day and night hauls, even those designed to fish as deep as 30 fathoms. To some extent, especially in summer, part of this variation is due to diurnal migration of animals away from the surface in the daytime, but recent work with high-speed samplers towed at 6 knots confirms that in the daytime macroplankton can escape from, or evade, the mouth of slow-moving nets, even those of 2 m diameter. The young fish trawl, it must be rembered, is preceded in the centre line by the warp, shackle and swivel, and then three rope bridles, while high-speed samplers have unobstructed entry in front. The earlier observations on the agile young clupeoids (Russell, 1926, 1928) illustrate well the defective catching power of the young fish trawl in daylight. Finally, the construction of the young fish trawl prevents it being turned inside out between stations, and since it is too big to be hosed down outside as with other gear, there is considerable 'spread' of plankton from one station to the next and the sorter has to have a very critical eye for damaged or 'hung-up' specimens.

In the absence of better samples one must make use of ring-trawl hauls. The inherent errors can be minimized in several ways: by treating each cruise separately, or by analysing only the work of one ship during a short time; by plotting contours at intervals of more than × 10. When choice is possible somewhat better numerical evidence can be got by hauling the ring trawl vertically instead of obliquely; this reduces differences in towing speed and vertical migration.

However, since possible errors are so large, no great accuracy is claimed for the results given below. Wherever possible I avoided tedious counting by subsampling. Successively larger samples were taken (the smallest with Stempel pipettes) and viewed in a squared dish under a low-power binocular microscope, until about 10 or more individuals had been counted. The actual counts, converted to total number per haul, are shown on the charts, with contours plotted at intervals of × 10 or more, but it must be borne in mind that the actual distribution may be more or less irregular than that shown.

In discussing the practical use of indicator species for distinguishing different bodies of water Russell (1939) has remarked that though probably many species form the characteristic association of plankton accompanying one or two more striking animals, it is the latter that should be selected as indicators,

for the rapid examination of large numbers of collections. Many species, both holoplanktonic and mesoplanktonic, have been suggested as 'indicators' by Russell (1935, 1936, 1939) and by other workers (Fraser, 1952; Glover, 1957; see also Abramova, 1956). In this and subsequent papers Russell's precepts have been followed with one or two exceptions. Thus I regard it as essential that an indicator species such as *S. elegans* should be present in all the samples from a supposed 'elegans' water area; and I have not been able to place so much reliance on medusae, which may be damaged in high-speed sampling and are often too scarce to be taken unless the large filtering capacity of the oblique 2 m ring trawl haul is employed.

In the case of the ring trawl samples described here, practically all the organisms are indicator species, and have been identified. The exceptions were young fish; euphausids; some copepods; certain medusae; and the oceanic siphonophores. These forms constitute a very small part of the daytime catches taken in the ring trawl off Plymouth.

THE 1955 SAMPLES

In the early months of 1955 sea-water samples taken at station E 1, 20 miles S.S.W. of Plymouth, were found to contain more phosphate-phosphorus than in any year since 1929 (Armstrong, 1957). Dr Cooper made a cruise in March, to try to trace the source (if any) of this nutrient-rich water, and worked a number of stations with the 2 m ring trawl in addition to a chemical programme (see Fig. 1 A). The following month part of the same area in the Western Approaches was worked by Dr Steven, assisted by Mr L. B. Pradhan, also with the 2 m ring trawl. Because of the possible significance of the high phosphate levels (cf. Kemp, 1938; Russell, 1939), I have examined the hauls from these two cruises and have compared them with some of the routine hauls taken at E 1 and the Eddystone throughout the same year.

In describing some of the chemical results of Dr Cooper's cruise, Armstrong (1957) remarks 'although notably high phosphate concentrations were found in surface waters over the continental slope south-west of Ireland, there is no evidence that water from there has ever entered the English Channel, and no satisfactory source for the high phosphate water was found within the area surveyed'. This was evidently Dr Cooper's opinion at the time (Report of Council for 1955–56, Vol. 35 of this *Journal*, p. 653). The plankton results, however, allow us to suggest an origin for both the 'western' plankton and the high phosphate water, and thus emphasize the usefulness of biological indicators in comparison with the cruder chemical methods available for distinguishing water masses.

The distribution of the commoner indicator species at the cruise stations is given in Figs. 1 and 2, while the seasonal variation of these animals off Plymouth is presented in Table 1. The more interesting changes and distributions are described on pp. 22 and 25.

TABLE 1. ZOOPLANKTON INDICATOR SPECIES, 1955

(Daytime catches made with the 2 m stramin ring trawl off Plymouth: ½ h oblique hauls. 'Eddystone': 2 miles east of Eddystone light; E1: 50° 02′ N., 4° 22′ W.)

Date	Station	Species											
		Aglantha digitalis	*Liriope tetraphylla*	*Nanomia* sp.	*Muggiaea* sp.	*Tomopteris* sp.	*Sagitta elegans*	*S. setosa*	*Candacia armata*	*Euchaeta hebes*	*Themisto gracilipes*	Larvae of Decapoda	Pilchard eggs
12. i.	Eddystone	—	80	—	—	—	44	1,300	—	—	—	—	—
18. i.	Eddystone	—	24	—	—	1	68	988	—	—	—	12	—
16. i.	Eddystone	—	10	—	5	28	292	1,892	4	—	—	—	—
16. ii.	E1	1	1	—	4	48	104	1,324	1	—	2	—	—
1. iii.	Eddystone	2	32	*	—	8	64	1,304	24	—	4	*	—
15. iii.	E1	1	—	*	—	60	1200	16,240	1680	—	—	*	—
30. iii.	Eddystone	—	—	—	—	20	40	3,270	—	—	—	*	—
9. v.	E1	—	—	—	—	—	2	3	12	—	—	384	8150
11. v.	Eddystone	—	—	—	—	—	—	16	—	—	—	1200	576
13. vi.	E1	—	—	—	—	—	—	10	—	—	—	*	4500
11. viii.	E1	—	200	—	576	—	—	12	—	—	—	832	—
15. ix.	E1	—	200	—	*	—	—	40	1	4	—	384	1920
17. xi.	E1	—	5	—	2900	—	4	1,730	2	4	—	576	480
21. xii.	E1	—	20	—	1060	—	7	376	—	—	—	144	—

* Present, not counted.

January

At the start of 1955 the plankton off Plymouth was not much different from other recent years (Russell, 1947; Corbin, 1948, 1949). The presence of *Liriope* suggests the remains of a south-western fauna of the previous autumn, but a small number of *Sagitta elegans* and *Tomopteris* indicated some western influence.

February

The following month the western component was much more marked, the significance of the increased numbers of *Tomopteris* and *S. elegans* being strengthened by the appearance of *Aglantha*: by this time the phosphate content exceeded 0·6 μg at./l. at the surface.

March

Aglantha was still present in March, and the phosphate level remained high. The disappearance of *Liriope* and *Muggiaea* left the western forms dominating the plankton, apart from continued numbers of *S. setosa*. From the maps (Fig. 1), it is clear the oceanic forms such as the salps were present only to the south-west, over the La Chapelle Bank (stations 3 and 9) while the more or less oceanic form *Sagitta serratodentata* was common at most of the stations along the edge of the continental shelf. The limits of this species may be compared with Corbin's results, for example April 1938 (Corbin, 1947, fig. 11 D): the area of dominance seems to have extended appreciably farther to the east. *S. setosa* was very much confined to the eastern stations. The 'western' indicators shown in Fig. 1, all have somewhat similar distributions. An area of abundance in the west (stations 10, 13 or 14, according to species) was partly separated by an area of scarcity (stations 8 and E 3) from the English Channel (Eddystone, E 1 and E 2) where the species were again abundant. The phosphate (Armstrong, 1957) showed a similar distribution pattern.

It may be noted that the presence of an oceanic component at stations 3, 4, 7 and 8 to judge from the plankton, appears to have had no connexion with the distribution of *Sagitta elegans*. This species occurred in large numbers in association with *S. serratodentata* only at the more northerly stations.

April

There were no samples from the Plymouth stations in April, but on 30 March, at the Eddystone, the western forms were obviously declining. In addition to a small percentage of *Sagitta elegans* only *Tomopteris* was present. The network of stations to the west was closer than in March and the distribution can be shown in more detail (Fig. 2). Among the oceanic forms *Rhincalanus* and *Sagitta serratodentata* were present along the edge of the continental shelf, while salps were still restricted to the most southerly deep-

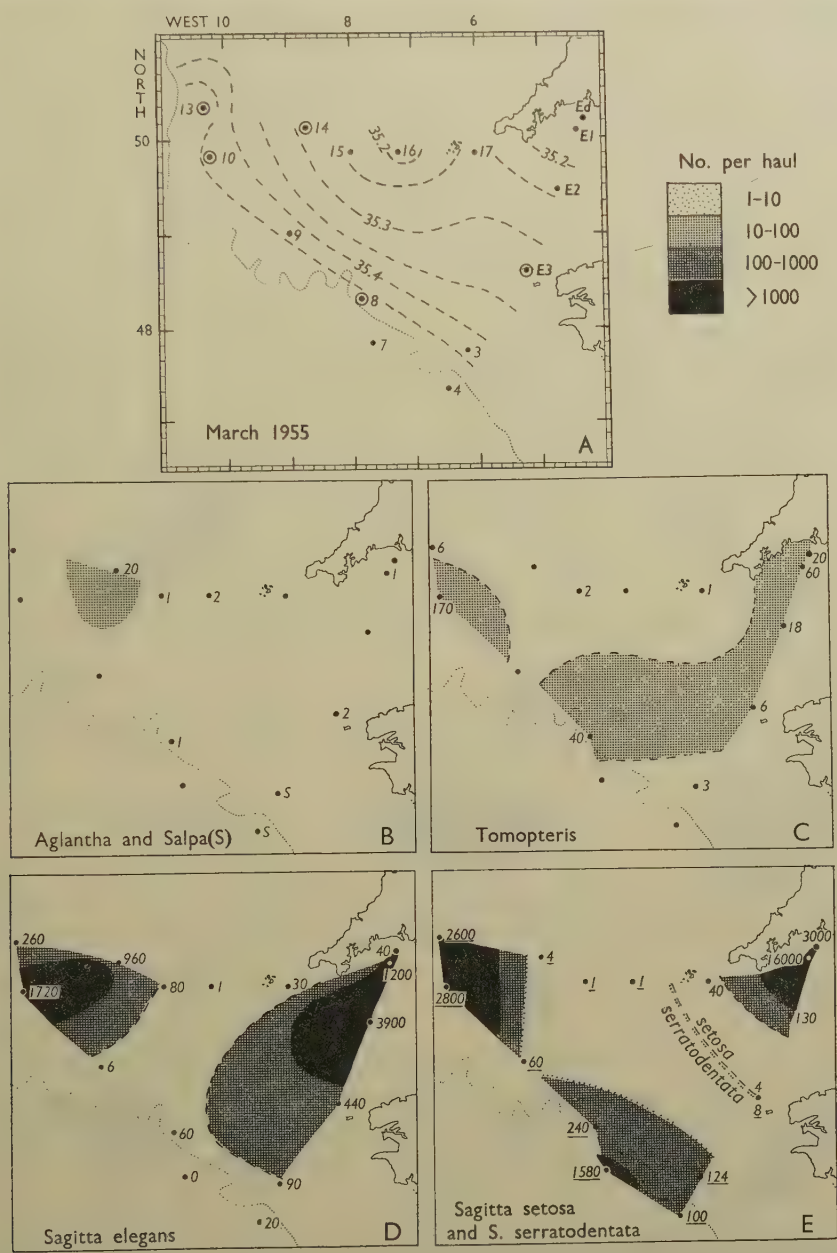

Fig. 1. Distribution of plankton and surface salinities 14–20 March 1955, with station at the Eddystone (Ed) on 30 March: A, position of stations (night hauls marked with a double circle) with approximate salinity contours at 10 m depth based on these stations and on others worked by Dr Cooper; B, *Aglantha digitalis* and *Salpa* spp; C, *Tomopteris* spp. (mostly *T. helgolandicus*); D, *Sagitta elegans*: E, *S. serratodentata* (to the west, numbers underlined) and *S. setosa* (to the east). The contour shading for this and subsequent maps is shown.

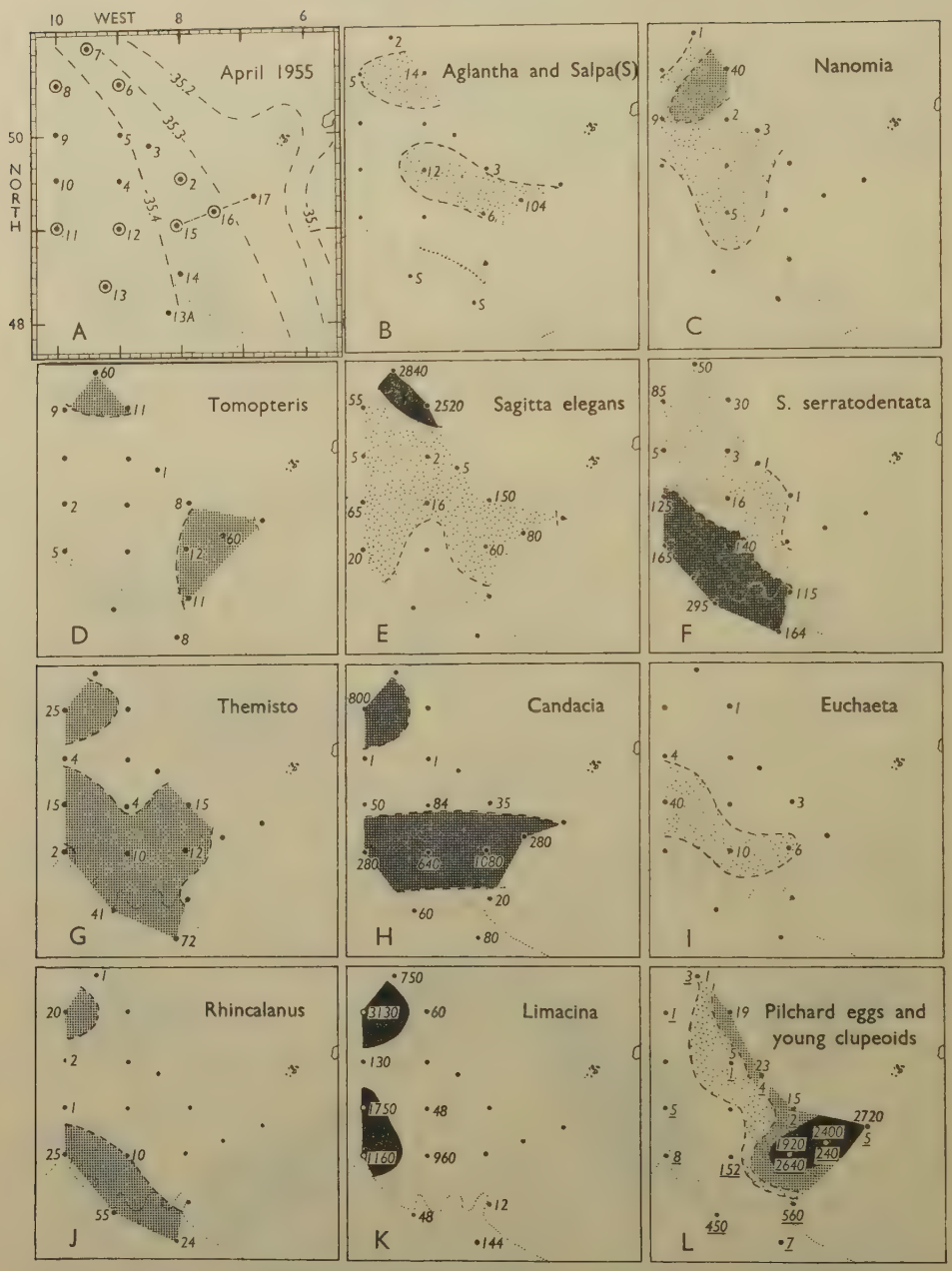

Fig. 2. Distribution of plankton, 8–12 April 1955: stations 15, 16 and 17 (joined by a dotted line on map A) were worked on 27–28 April. A, station positions (night hauls shown by double circle) with salinity contours taken from the International Council Synoptic Hydrographic charts (Smed, 1955) showing the surface monthly mean: *B*, *Aglantha digitalis* and *Salpa* spp; C, floats of *Nanomia* sp.; D, *Tomopteris* spp. (mostly *T. helgolandicus*); E, *Sagitta elegans*; F, *S. serratodentata*; G, *Themisto gracilipes*; H, *Candacia armata*; I, *Euchaeta hebes*; J, *Rhincalanus* sp.; K, *Limacina retroversa*; L, eggs of *Sardina pilchardus* (contoured) and young clupeoids (figures underlined).

water stations. The 'western' indicators show a pattern comparable with that of March, particularly *Aglantha*, *Tomopteris* and *Candacia*. The centre of abundance of *Sagitta elegans* would appear to have shifted farther north: south of the 50° parallel it exceeded 100 per haul only at one night station. A similar northern centre is suggested for *Nanomia*, and neither species seems to have any relationship with the oceanic component. *Themisto* and *Euchaeta*, from their geographical distribution, as far as it is known, are southern forms, and the patterns shown in Fig. 2 are probably falsified by the absence of stations off Brittany. In March they were present at most stations, without a clear pattern and have not been shown in Fig. 1. Russell (1935) regarded *Themisto* as a 'western' form, but recent investigations with the high-speed samplers show it as both a western and south-western form at times. He identified *Euchaeta* with 'south western' or 'Biscay water', but the present report shows that it now penetrates into the preserves of western forms (see p. 31). The distribution of the remaining southern form, *Sardina pilchardus*, is shown as eggs and post-larvae, and the pattern suggests a north-eastward progression of spawning. In March, pilchard eggs were taken at some of the more southerly stations (E3, 3 and 9; over 100 at the latter two) and together the two 1955 cruises indicate a possibly earlier start and more northerly extension of the spawning area compared with 1937–9 (cf. Corbin, 1947).

May and June

The last traces of western forms disappeared from the Plymouth stations in May, to be replaced by the usual dominance of pilchard eggs.

August and September

The pilchard dominance continued into the autumn. In August the usual appearance of south-western forms (Russell, 1935) occurred, and both *Liriope* and *Muggiaea* remained in the Plymouth area to the end of the year. They seem to have been accompanied by some *Candacia* and *Euchaeta* in September and November.

November and December

The numbers of *Liriope* were reduced at the end of the year, when slight signs of western influence were suggested by the reappearance of *Sagitta elegans* in small numbers. The numbers of all *Sagitta* increased at this time, coincident with the cessation of pilchard spawning.

1957

Two cruises were made to the west of Plymouth in 1957, vertical hauls being taken with the 1 m net in May, and with the 2 m net in September. Other work restricted the programme in May, and bad weather curtailed the later cruise. On both trips additional samples were taken with horizontally towed

TABLE 2. ZOOPLANKTON INDICATOR SPECIES, 1957

(Daytime catches made with the 2 m stramin ring trawl off Plymouth: ½ h oblique hauls.)

Date	Station	*Liriope tetraphylla*	*Muggiaea* sp.	*Nanomia* sp.	*Tomopteris* sp.	*Sagitta elegans*	*S. setosa*	*Candacia armata*	*Euchaeta hebes*	Other copepods	*Themisto gracilipes*	Pilchard eggs	Other fish eggs	Young clupeoids	Other young fish	Decapod larvae
1. i.	Eddystone*	—	—	—	13	—	1830	—	—	170	—	—	23	2	2	135
24. i.	E 1	—	33	1	—	18	1140	—	—	60	—	—	90	8	2	120
19. ii.	Eddystone	1	15	—	—	90	900	7	—	6	—	1	90	63	9	690
5. iii.	E 1	30	12	†	—	183	180	9	—	58	1	21	1410	—	—	1830
11. iii.	Eddystone	—	3	—	1	—	102	6	—	21	—	—	150	105	15	780
18. iii.	Eddystone‡	—	—	—	—	—	—	(27)	—	(21)	—	(45)	(99)	—	—	—
1. iv.	Eddystone	—	—	—	—	—	—	—	—	660§	—	11,100	60	180	126	2280
24. iv.	E 1	—	—	—	—	15	—	54	—	840	—	6,000	240	—	90	7560
9. v.	E 1	—	4	—	—	—	300	8	—	800	—	6,560	80	32	76	4500
12. vi.	Eddystone	—	†	—	—	—	192	—	—	1500	—	10,560	120	180	54	780
25. vi.	Eddystone	—	—	—	—	—	360	—	—	180	—	5,460	60	45	45	3000
16. vii.	E 1	—	†	—	—	—	234	—	—	180	—	1,620	450	30	—	180
21. viii.	E 1	—	2400	—	—	—	720	3	—	180	—	180	36	162	30	1140
27. viii.	Eddystone	—	1890	—	—	—	30	—	—	252	—	15	24	9	9	480
17. ix.	E 1	—	1800	—	—	—	12	2	1	12	—	1,260	—	3	18	360
9. x.	Eddystone	—	360	—	—	—	306	5	—	132	—	252	—	15	—	1200
25. x.	Eddystone	—	900	—	—	—	120	3	—	45	—	25	5	96	3	282
6. xi.	E 1	—	240	—	—	—	600	48	13	42	—	30	3	150	—	12

* Night haul. † Present. ‡ Not well preserved. § Includes 600 *Anomalocera patersoni*.

high-speed samplers based on the Hardy indicator (Glover, 1953) but it has been found impossible to relate the catches of these small samplers with the vertical hauls. The volume of water filtered was of the order of 1 or 2 m^3, and thus several of the larger 'indicator' species were not taken at all, others being distributed erratically. The samplers were run day and night at only 10 fathoms depth, the limit of the equipment at the time, and their catches appeared to be affected by diurnal migration.

The results of the May cruise are shown in Fig. 3, and Table 3 gives the results for the September cruise. In Table 2 are presented the main species found in the oblique hauls of the 2 m ring trawl off Plymouth throughout the year.

January to March

In the spring of 1957, the Plymouth samples showed a trend comparable to that in the spring of 1955. Some 'western' indicators were present in January and February, accompanied by some 'south-western' forms. At the beginning of March *Sagitta elegans* was found in some numbers, but no further increase in western forms took place.

April

Except for the sample at E1 on 24 April, *Sagitta elegans* was not present off Plymouth for the rest of the year. Pilchard spawning began earlier than in 1955, the surface temperatures at E1 in February, March and April being 1 to 2° higher than in 1955. The activity of the pilchard and the higher temperatures may have had an effect on the numbers of *Sagitta elegans* and *Candacia*, but the absence of *Aglantha* suggests that there had been very little communication with the apparent source of the 'western' forms.

May

The results of the cruise in May appear to confirm the presence of a 'western' component in the Channel, for though by then absent off Plymouth, some western forms were found at the stations near and south of the Lizard (1 and 2). Apart from this area there was a generally reciprocal distribution of 'western' and 'south-western' forms. Species such as *Aglantha*, *Tomopteris* (excluding the unidentified oceanic species at stations 7 and 25), *Sagitta elegans*, *Candacia* and *Limacina* were all most abundant along the northern and western line of stations, while *Liriope*, *Muggiaea*, *Euchaeta* and pilchard eggs occurred only at, or were most numerous at, the southern stations. The oceanic forms, salps, *Rhincalanus*, *Sagitta serratodentata* and certain species of *Tomopteris* were found only at the extreme south-western stations. *Sagitta setosa* was restricted to the northern side of the Channel, but was in fact most abundant at the stations north of Scilly, where *S. elegans* was also abundant.

Compared with March and April 1955, May 1957 showed a closer approach to the area of abundance of the 'western' fauna to the Isles of Scilly; there was no region of poor macroplankton just to the west as in 1955.

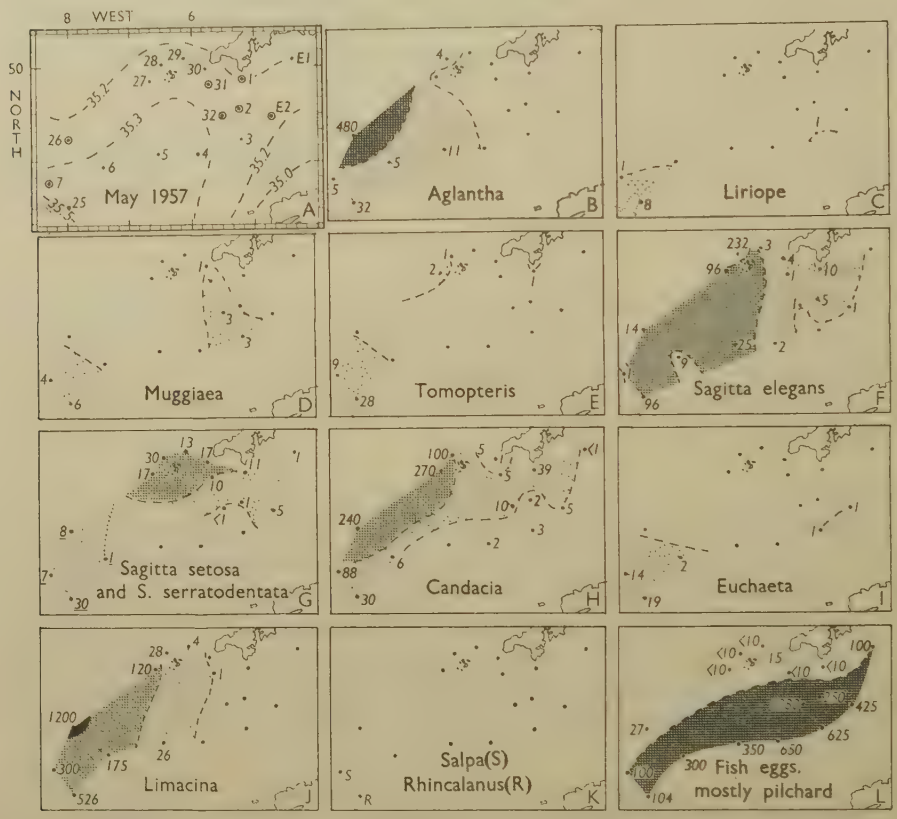

Fig. 3. Distribution of plankton and surface salinity, 1–9 May, 1957. A, position of stations, night hauls shown by double circles, and salinity contours at 0–10 m, derived from the International Council charts (Smed, 1957) modified by samples taken on the cruise by Mr F. A. J. Armstrong; B, *Aglantha digitalis*; C, *Liriope tetraphylla*; D, *Muggiaea* spp., probably all *M. kochi*; E, *Tomopteris* spp.; F, *Sagitta elegans*; G, *Sagitta serratodentata* (to the west, figures underlined) and *Sagitta setosa* (to the north); H, *Candacia armata*; I, *Euchaeta hebes*; J, *Limacina retroversa*; K, *Rhincalanus* sp and *Salpa* spp.; L, fish eggs, more than 95 % *Sardina pilchardus* at stations with over 100. For this cruise, with vertical nets, the numbers given are those per haul, i.e. in the column of water below a circle of 1 m diameter (ca 0·785 m²) to within 10 m of the bottom.

April–July

Pilchard dominated the Plymouth plankton hauls for the summer, until the appearance of 'south-western' forms again in August.

August–November

Liriope was not found in the autumn but *Muggiaea* and *Euchaeta* appeared, again accompanied by small numbers of *Candacia*. Pilchard spawning continued to the end of the season's sampling and 'western' forms did not reappear in the area until March the following year (to be reported upon in a later paper). On the curtailed cruise in September, however, the 'western' forms *Aglantha*, *Tomopteris* and *Sagitta elegans* were found at station 2, 30 miles south of the Lizard (Table 3). Presumably this influence did not reach Plymouth for 6 months, or else it died away without travelling much farther eastwards.

TABLE 3. ZOOPLANKTON INDICATOR SPECIES FOUND DURING THE SECOND PLANKTON CRUISE IN 1957

(Vertical hauls with 2 m stramin ring trawl, 10 September.)

Station and depth of haul	*Aglantha digitalis*	*Muggiaea sp.*	*Tomopteris helgolandicus*	*Sagitta elegans*	*S. setosa*	*Candacia armata*	*Euchaeta hebes*	*Themisto gracilipes*
(1) 49° 55′ N., 5° 11′ W. from 40 f.	—	—	—	2	83	32	—	—
(2) 49° 27′ N., 5° 9′ W. from 50 f.	12	—	5	88	360	45	5	8

E 1 and Eddystone oblique hauls during same period are given in Table 2.

The series of samples off Plymouth in 1957 illustrate how deceptively unchanging the plankton at the Eddystone may appear compared even with that at E 1, 10 miles to the south. As the cruises show, the latter station itself is by no means representative of the northern half of the Western English Channel.

POSSIBLE MOVEMENTS OF PLANKTON AND WATER MASSES

There is great danger in trying to attribute some meaning to the limited number of samples taken in 1955 and 1957. However, some tentative suggestions as to the course of events, both biological and hydrographical, can be made.

In the spring of 1955 a macroplankton community containing some 'western' indicators, and accompanied by a characteristic water mass with high winter phosphate levels appeared in the Western Channel. The stations worked in the Western Approaches suggest that the plankton and water mass may have been derived from an area to the south of Ireland, i.e. to the north and west of the entrance to the English Channel. Distribution of surface salinities (10 and 0 m) suggests that a typical cyclonic swirl (cf. Matthews, 1914; Harvey, 1929) occupied most of the area south of Ireland to the latitude of Scilly, in March and April. The 'western' body may have been drawn

in as part of this swirl at some preceding time, but as communication had been partly severed at the time of the cruise, it is not possible to say when and along what parallel it entered the English Channel. It is worth noting that the survival and spread of these 'western' forms, which are all of north Boreal character, may have been favoured by the cold summer of 1954, and winter of 1954–5, when the surface temperatures observed at E1 were among the lowest since the 'twenties. Moreover, the autumn of 1954 was marked by a succession of westerly winds which would be expected to strengthen the southern track of the cyclonic swirl. The more normal southerly and easterly winds of recent years did not supervene until the early spring of 1955; they may well have produced the separation observed in March.

After April 1955 the 'western' component soon disappeared, and there appeared to be no local benefit from the extra phosphate which may have entered the Channel.

In the spring of 1957 a cyclonic circulation was also present, to judge from the salinities in May. It appears, however, that the plankton had less southerly spread from the Irish region and that fewer 'western' forms were drawn into the Channel from the north-west.

Origin of 'western water'

From his own material taken near Plymouth, supplemented by samples from French and Irish stations farther west, Russell (1935, p. 315) deduced that his 'western water' originated from a source to the south of Ireland. The 1955 and 1957 samples are entirely consistent with this view, and, as far as it goes, the chemical evidence is also in agreement. Thus, the 'western water' is to be regarded rather as 'north-western' water, and, therefore, not now in the path of what might be thought to be the normal inflow into the English Channel from the west.

Neither the earlier work nor the recent investigations provide any evidence that the source of the 'western water' is a result of upwelling of deep, nutrient rich water along the edge of the continental shelf (for hypotheses see Russell, 1935, p. 322; Cooper, 1955*a*, *b*). Nevertheless, if upwelling does occur, a study of the bottom topography between France and Ireland suggests that it is more likely to occur, or have more effect, in the area just to the south of Ireland. On recent cruises of R.V. 'Sarsia' I have confirmed the difference shown on Admiralty charts and by Day (1959): the shelf from 49° N. to the Irish coast is bounded by long, relatively smooth slopes (of sandy or oozy nature, difficult to dredge over), whereas the southern edge of the Celtic Sea has a steeper slope dissected by many deep canyons, with rock exposures bearing an abundant epifauna. The effect of internal waves in deep water 'beating' along the end of the shelf may be compared with heavy waves on an open coast. Against a steep cliff the water-level rises and falls comparatively little at each wave, and where the rock is broken the energy is dissipated as

spray. But on a smoothly sloping rocky ledge or beach the wave energy is translated almost completely to forward (and upward) motion and the water dashes a long way up the shore. By analogy then, we might suggest that upwelling of water from below 400 m would be more probable in the north Celtic sea, provided other factors were equal and that internal waves occurred. However, it is possible to explain the ecosystem found off the southern Irish coast in other ways, and of course, it does not follow, as some workers believe, that upwelling of water with a high phosphate content would necessarily produce the zooplankton community observed.

THE DISTRIBUTION OF *EUCHAETA HEBES*

The warm-water copepod *Euchaeta hebes* Giesbrecht was first recorded from the south-western entrance to the English Channel during the International Investigations. In November and February in some years it was found also though less abundantly, as far north as station E6 (Gough, 1905, 1907; Bygrave, 1911). According to Farran (1911) the species was not taken at the Irish stations in those years, and his chart of the limits of regular occurrence of the species based on Gough and Bygrave was used by Russell (1935) to illustrate the distribution of south-western water. The distribution in 1903–5

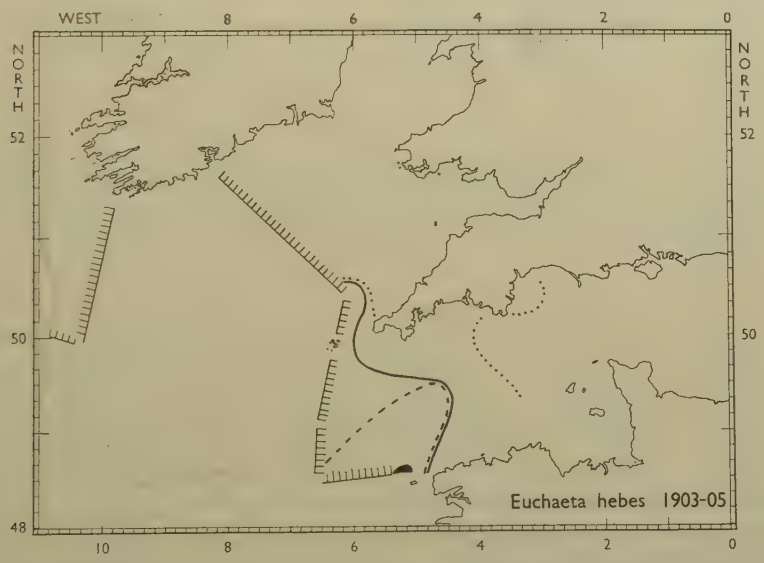

Fig. 4. The distribution of *Euchaeta hebes* in 1903–5, according to Gough (1905, 1907). The southern and western limits of the area investigated by the English and Irish Research vessels in 1903–11 is also shown. Small black area: species present at all seasons. Broken line: extreme northern limit found in summer (May and August) in certain years. Solid line: usuai northern limit in winter (November and February). Dotted line: extreme northern and eastern limit in winter in one year.

when the species reached its farthest north during the period 1903–11 is shown in Fig. 4.

If we accept the previous evidence, then in 1955 and 1957 *E. hebes* ranged over a much wider area of the English Channel and western approaches than it did 50 years earlier. This extension of range continued in later years (to be described fully in another paper). For example, in 1959 small numbers were taken as far north as Bardsey Island in the Irish Sea, and the same species was reported from more oceanic waters to the north of Scotland.[1] Some further evidence on the distribution of *Euchaeta* has been provided by the mackerel cruises. Mr P. G. Corbin has kindly allowed me to examine his records for 1937, when *E. hebes* was included among the animals counted, and the results are shown in Fig. 5, at stations listed by Corbin (1947). Even in 1937 *Euchaeta* occurred over a wider area than in 1903–8.

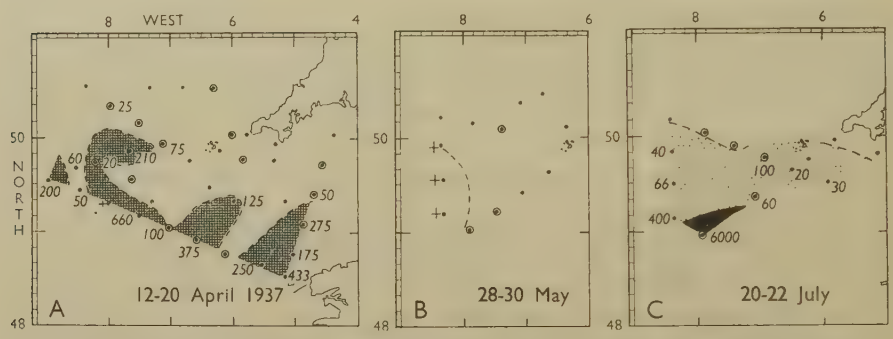

Fig. 5. Distribution of *Euchaeta hebes* in the spring and summer (April, May and July) of 1937, to compare with summer 1903–5. Numbers per ½ h oblique 2 m ring trawl haul, except B for which only presence (+) in 1 m net hauls is given. The station positions were given by Corbin (1947). Night samples, including dusk and dawn, are shown by double circles. A, contour drawn at 100 per haul; C, contours drawn at 10 and 1000 per haul.

This apparent extension of range of a warm water species gains greater significance when compared with the now well established rise in surface temperature in the area. From Smed's data (1952) the annual mean in the Celtic sea has risen to such a level that the waters just south of Ireland (International Council Area Q) now have on annual mean approaching that found in the 1900s much farther to the south (International Council area P), to which region *Euchaeta* was then apparently restricted (Fig. 6). Rises in surface temperature, especially of annual means, have also been shown for the Seven Stones (Bowden, 1955) and for station E 1 (Southward, 1960), and the trend is obviously applicable to the whole English Channel and western approaches.

The idea of an increased occurrence of warm-water species in the English

[1] Personal communication R.S. Glover.

Channel was first suggested by Russell (1939, p. 186), and confirmatory evidence has now accumulated (Russell, 1953). An advance of southern forms and retreat of northern forms has definitely occurred on the shore (Crisp & Southward, 1958). Similar changes connected with the general warming up seem to have taken place in other habitats, for example among the commoner demersal fishes (unpublished observations of the late G. A. Steven) as well as with the rarer pelagic forms mentioned by Russell (1953), and it would be surprising if plankton organisms were unaffected. It might be assumed, then, that the extension in range of *Euchaeta* is not an isolated occurrence, and that there might have been a general northward shift of distributional limits in the

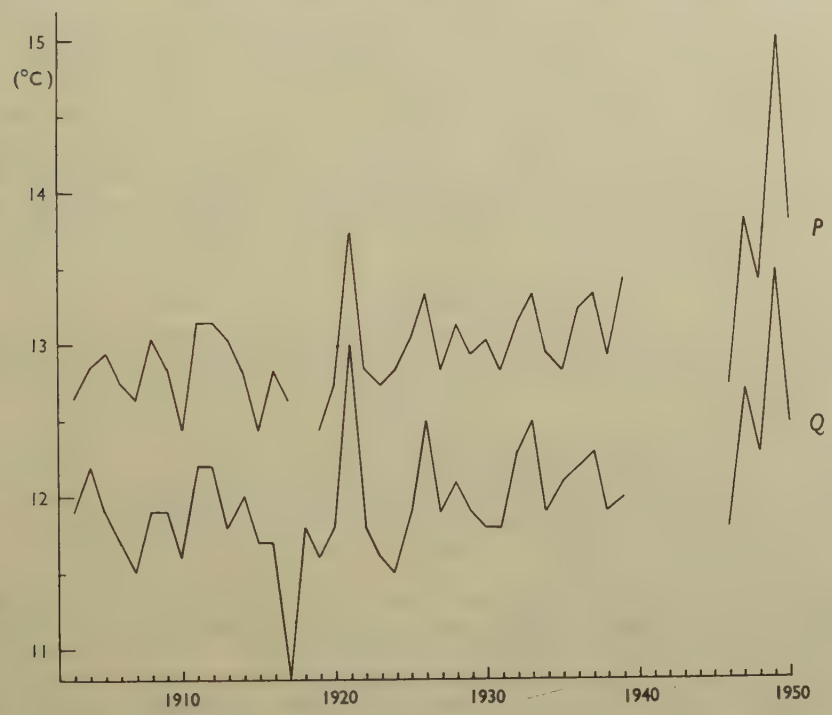

Fig. 6. Annual means of sea-surface temperature for International Council areas P (South Celtic Sea) and Q (north Celtic Sea, South of Ireland) 1903–50. Based on Smed (1952).

area. We may have, in the temperature change, a possible explanation, if only indirect, and in part, of the reduced occurrence off Plymouth since the 1920's of the western forms and the larger macroplankton species. Many of these (e.g. *Aglantha*, *Sagitta elegans*, *Meganyctiphanes*) are of northern origin and thus near their southern limits[1] at the entrance to the English Channel. Other factors, of course are involved; a full discussion will be given later.

[1] Excluding deep-water communities.

I am greatly indebted to Dr F. S. Russell for advice and discussions on this problem. Thanks are due to Dr L. H. N. Cooper and Mr A. D. Mattacola for the 1955 samples.

SUMMARY

Previous plankton work at Plymouth is reviewed briefly, and the limitations of the stramin ring trawl as a quantitative sampler discussed. The distribution of certain zooplankton 'indicator' species caught in hauls of the 2 and 1 m ring trawl during cruises in 1955 and 1957 is described in comparison with regular 2 m hauls taken throughout the same years at two stations near Plymouth.

The results support previous suggestions that 'western' water at Plymouth is derived from a region to the south of Ireland, i.e. from the north-west, and is not now in the direct path of the flow of water into the English Channel from the west.

An apparent northward spread of the warm-water copepod *Euchaeta hebes* during the past 50 years may be connected with the rise in sea temperature over the same period. It is suggested that related changes in distribution might be responsible in part for changes in the macroplankton community off Plymouth since the 1920's.

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THE EFFECTS OF SALINITY ON HERRING AFTER METAMORPHOSIS

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(Text-figs. 1–7)

Although in general the herring (*Clupea harengus* L.) is a species found offshore in waters of salinity close to 35‰, at certain stages of its life history it can be found inshore. In particular the immature fish up to the age of 2½ years can be found in river estuaries. The adult fish also come inshore to spawn, for example Ford (1929) records that ripe herring have been taken in the Tamar estuary, and Brandhorst (1959) reports that successful spawning took place in the Kiel canal in salinities down to 5‰. Bückmann, Harder & Hempel (1953) discuss the salinity tolerance of herring and give some values ranging from 1·7–12·2‰ for the spawning places of German coastal herring. Brawn (1960) found 5·2‰ was tolerated by Passamaquoddy herring. McMynn & Hoar recorded the presence of Pacific herring (*Clupea pallasii*) spawning in water of salinity 8·21‰.

Holliday & Blaxter (1960) showed that the eggs and larvae of the herring have an extremely wide salinity tolerance and are capable of maintaining the concentration of the tissue fluids within relatively narrow limits when subjected to a wide range of salinities. The experiments now described were a continuation of this work and were designed to test the salinity tolerance of the herring after metamorphosis and to investigate the physiological basis for this tolerance.

METHODS AND RESULTS

Salinity tolerance

The salinity tolerance of the following groups of herring was determined; 0-group, up to 1½ years old (9–15 cm) in length; 1-group, 1½–2½ years old (15–24 cm); and adult herring greater than 2½ years old (*ca.* 24 cm). The fish were maintained either in the aquaria tanks at the laboratory or in 50 l. glass carboys on board a research vessel. In the aquarium, low salinities were made up from aquarium sea water and tap water. On board ship sea water was used from the region in which the herring were caught and was diluted with fresh water from the ships' tanks. Where high salinities were required sodium chloride was added to sea water.

In all experiments the fish were kept in the particular salinity for at least 24 h. If 50% were alive at the end of this time then the fish were said to

tolerate the salinity. In some confirmatory experiments fish were held in water of low salinity for up to 12 months. The results are summarized in Fig. 1.

The lower limit of salinity tolerance for both immature and mature fish is very close to 6·0 ‰ and the upper limit lies between 40 and 45 ‰. The significance of the rather high mortalities at some of the intermediate salinities within the tolerance range is probably due to variations in the descaling of the fish caused by handling and a tendency to choose the fish in best condition for experiments at the extremes of salinity.

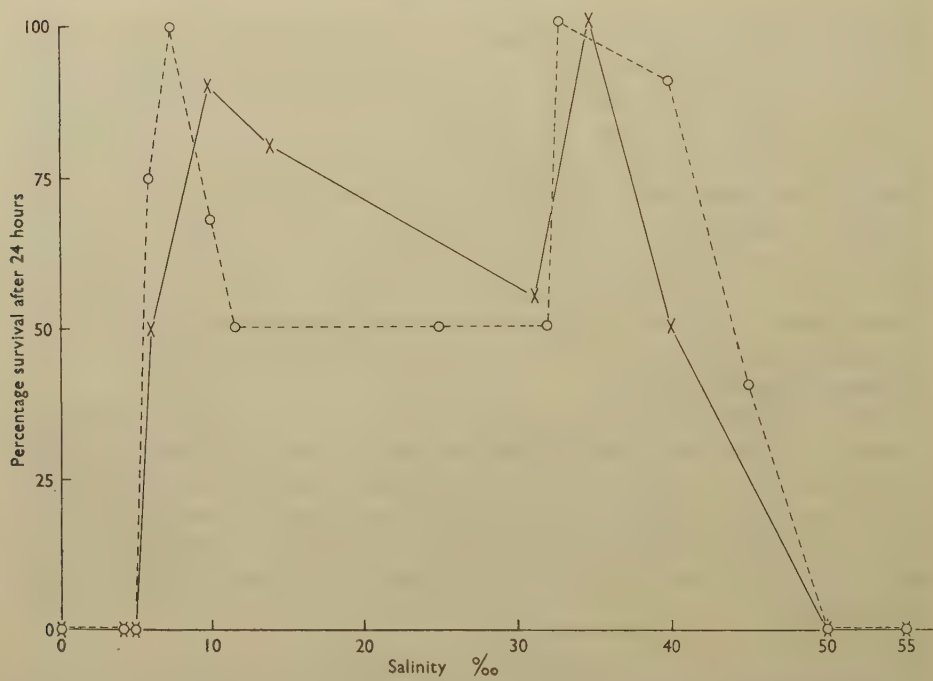

Fig. 1. Survival of herring transferred from sea water (32–34 ‰) to different salinities: O- - -O, Fish 24–30 cm in length; × - - - ×, fish 9–24 cm in length.

Weight changes

To obtain more details of the effects of different salinities on herring, in particular in relation to the osmotic passage of water into and out of the fish through the semi-permeable membranes, the weight changes of fish as they were transferred from one salinity to another were measured.

Weighings were made to the nearest 0·5 g. Three methods were used; on board ship the fish were placed in a muslin bag slung from a spring balance; in the laboratory a special weighing box was used in which the fish were weighed in water. As confirmation of some of the results and when weighing

small herring the fish were anaesthetized with a 1/20,000 solution of tricaine methane sulphonate and accurately weighed on a chemical balance in air.

Fig. 2 illustrates the results obtained. It is obvious that a rapid gain of weight follows the transference of herring from sea water to water of lower salinity. In salinities above the tolerance level (6‰) regulation starts usually within 12 h and the weight commences to fall. In waters below 6‰ regulation is inadequate and even at 20 h, which was the longest period at which a fish survived in these salinities, no decrease in weight was evident, the weight gain continuing until death. When fish were transferred to salinities greater than sea water there was a weight loss of about 7%, recovery taking place in 40‰, but not in 45 or 50‰ (both above the upper tolerance level).

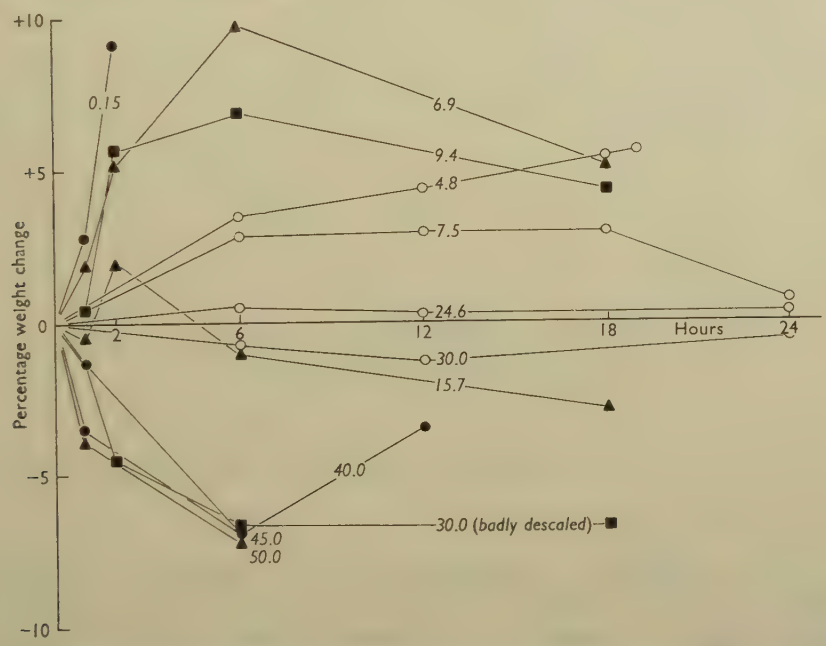

Fig. 2. Change in weight of herring transferred from sea water (32–34‰) to different salinities. Solid symbols: fish 9–12 cm in length; open symbols: fish 17–24 cm in length.

The results obtained when fish were transferred from water of a low salinity (10·2‰), where they had been living for 9 months, to sea water, are shown in Fig. 3. There is an initial weight loss, probably due to a time lag before the regulatory mechanisms changed over to the sea-water level.

The weight changes recorded in living herring when transferred from water of one salinity to water of another salinity were of the same order as those found by Sumner (1905) and Black(1948) in *Fundulus*. In some instances the fish seemed to overcompensate and in consequence there was some oscillation around the original weight before it was finally reached. The variability in

some of the results, particularly the losses in weight when fish used as controls were transferred from sea water to sea water, was probably due to two factors. First, there was osmotic loss due to increased permeability of skin after being handled—scales were invariably lost no matter how carefully the fish was transferred. Secondly, and more important, laboratory (handling) diuresis probably occurred. This is a phenomenon due to shock which results in an increased flow of urine and hence a loss of water and some salts. In these experiments all fish were handled to the same extent and it must be assumed that this diuresis was of the same order in all the fish.

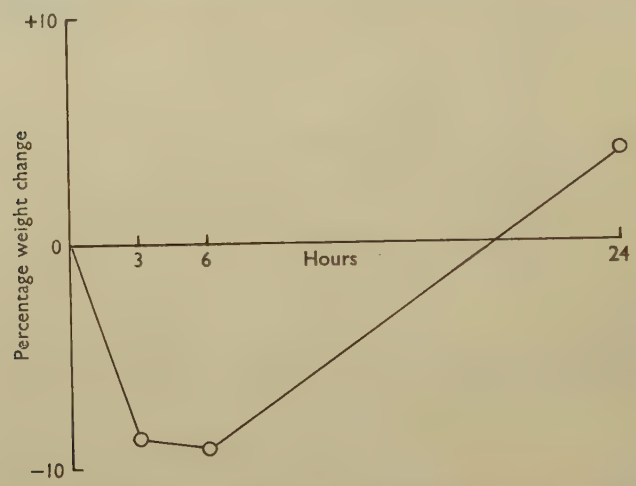

Fig. 3. Change in weight of fish transferred from salinity 10·2 ‰ to sea water (32–34 ‰).

Osmotic concentration of the blood

In order to measure the changes taking place in the blood of the herring subjected to different salinities, samples of blood were taken at intervals and the freezing-point obtained. The difference, known by the symbol Δ, between this and the freezing-point of water (0° C) is a measure of the blood concentration.

Samples of blood were taken from the heart by means of a silica-glass capillary. The herring is very delicate and never survived this, therefore a fish was sacrificed for each sample and different fish were used from the weight-change experiments. The determinations of the freezing-points were made on Ramsay & Brown's (1955) apparatus.

The change in osmotic concentration with time is shown in Figs. 4 and 5 for herring 9–24 cm long transferred from sea water to lower and higher salinities. The mean value of Δ for fish living in sea water was 0·95, i.e. −0·95° C (equivalent to a salinity of 15·8 ‰). This value is, incidentally,

higher than the published values for other teleosts, e.g. Black (1957) gives 0·78 as an approximate value for the blood of marine teleosts.

It can be seen from Fig. 4 that when herring are transferred from sea water to salinities which are lower than that isotonic with the blood (i.e. 15·8‰),

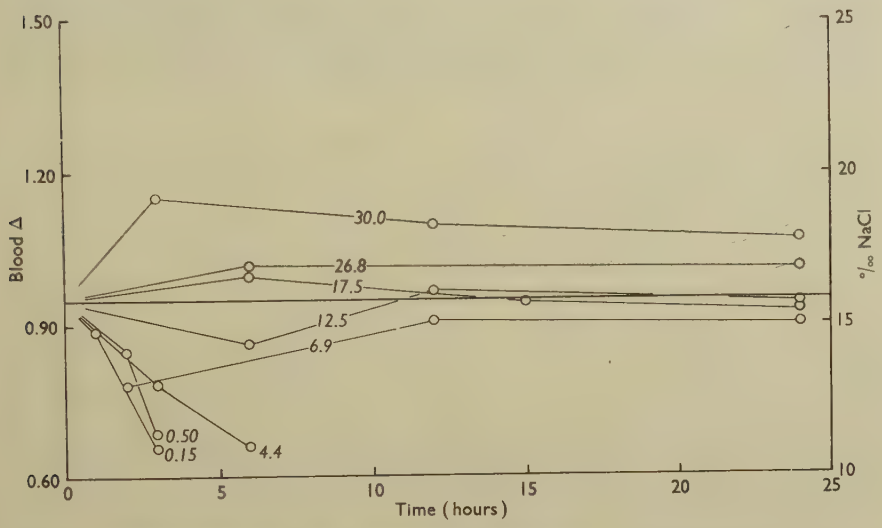

Fig. 4. Osmotic concentration of the blood of herring (9–24 cm) transferred from sea water (32–34‰) to lower salinities.

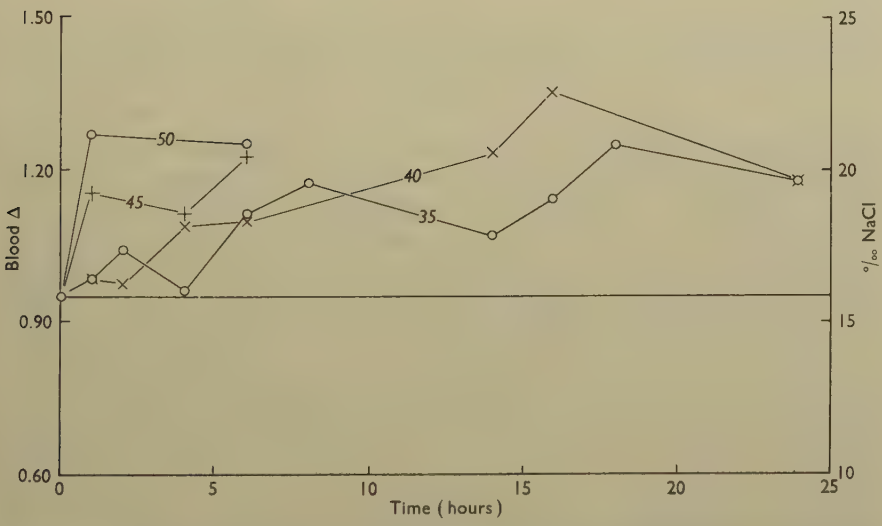

Fig. 5. Osmotic concentrations of the blood of herring (9–24 cm) transferred from sea water (32–34‰) to higher salinities.

there is an immediate fall in the blood concentration. Provided these salinities are not below the lethal level of 6‰ a recovery takes place, the osmotic concentration returning to near the normal level. Below 6‰ there was no such recovery.

Where herring were transferred from sea water to higher salinities (see Fig. 5) there was an increase in blood concentration. In salinities up to 40‰ a recovery started to take place, but in salinities of 45‰ and higher the rise was rapid and there was no recovery.

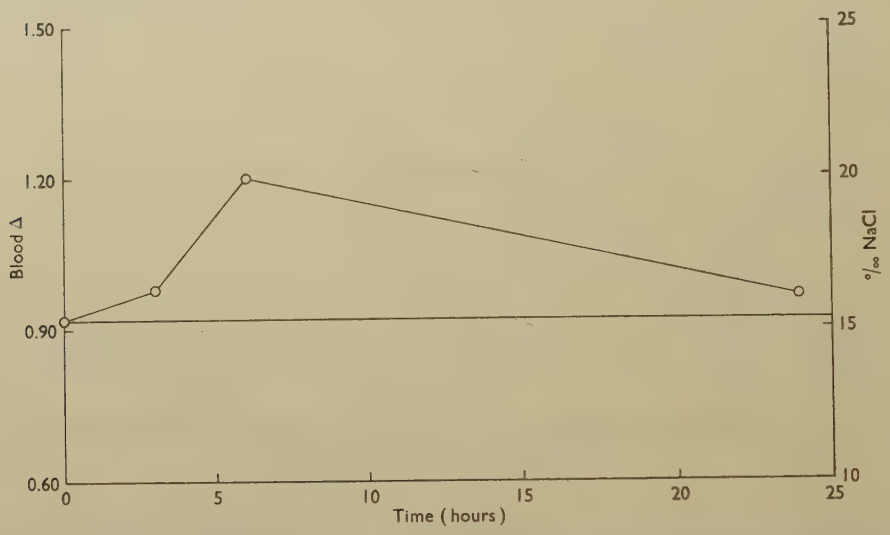

Fig. 6. Osmotic concentrations of the blood of fish (15 cm) transferred from 10·2‰ to sea water 32–34‰.

The results in Fig. 6 show the changes that took place when the fish (15 cm long) were transferred from 10·2‰ to sea water. There was an initial rise in blood concentration which was later reversed. The difference in time relationships between this rise and the weight changes (see Fig. 3) is probably due to the fact that two different groups of fish were used. The blood Δ of the fish that had spent 9 months in water of salinity 10·2‰ was 0·92 (≡15·3‰) compared with 0·95 (≡15·8‰), the average value for fish living in sea water.

Badly descaled herring in sea water showed large increases in blood concentration with values of from 1·25 to 1·65 before death.

It is evident that the herring is capable of tolerating and regulating body fluids within a wide range of salinities. A study was therefore made of the organs and tissues involved in the regulating processes to see how they reflected this ability.

The kidney

Kidneys were taken from fish of varying length and maturities living in different salinities and fixed in either Zenker or formol saline. Five specimens were serially sectioned at 5 μ and every 100th section mounted. In this way the general structure and distribution of tubules and glomeruli was found. Sections were taken from particular areas in many other specimens. They were stained with Mallory's triple stain or haematoxylin and eosin and a few with Masson's trichrome stain.

The kidneys in the herring are fused along most of their length and there is no visible demarcation in section. Glomeruli are present throughout the whole kidney except for the first and last few millimetres. The greatest concentrations are from the anterior head kidney to the mid-region, the numbers diminishing posteriorly. There is a random distribution dorso-ventrally. There appears to be no significant difference in number or distribution of glomeruli between the two kidneys.

In section the Malpighian bodies appear as large structures with a good blood supply within the capsule. The diameter in section of the capsules in fish both 20–30 cm and 6–12 cm long is 50–70 μ. The glomerulus has a mean diameter of 35 μ. From the capsule an open, ciliated short neck segment leads to the first part of long proximal convoluted tubule. There is an abrupt transition from this to the second part of the proximal convoluted tubule, which is shorter and leads to the collecting tubule. There is no distal tubule. The blood supply to the tubules is extremely good, particularly to the first part of the proximal tubule.

In view of the wide distribution and large numbers of glomeruli in the kidney, estimates of the total number of glomeruli per fish were made. The technique used was that of Nash (1931). The kidneys were dissected out and homogenated after treatment with hydrochloric acid. The larger kidneys were then made up in a suspension of water to 100 ml., the smaller ones made up to 25 ml. Ten subsamples of 0·5 ml. were taken from the 100 ml. suspension and five 0·5 ml. samples from the 25 ml. suspension. The glomeruli were counted in a squared dish under a binocular microscope.

The results are expressed in Fig. 7 which shows, first, glomerular count in relation to body length and, secondly, glomeruli per square centimetre of surface area of the fish. It seems that the smaller fish have fewer glomeruli than large fish but they have more glomeruli per unit of surface area.

Comparing these results with those of Nash, it is evident that the herring has a high glomerular count, resembling strongly the freshwater teleost.

Salt regulation

It has been established that salt is excreted in the head region of fish (Keys, 1931), and Keys & Willmer (1932) suggested this was carried out by

certain large acidophilic cells on the gills (the so-called chloride secretory cells).

The occurrence of these cells was studied in 0-group, 1-group and adult herring by fixing gills and buccal epithelium in Zenker, Bouin or formol saline, sectioning at 3–8 μ and staining with haematoxylin, eosin and alcian blue. The acidophilic cells were found in small numbers on the gills, buccal epithelium and on the inside of the operculum but they were found in by far the greatest

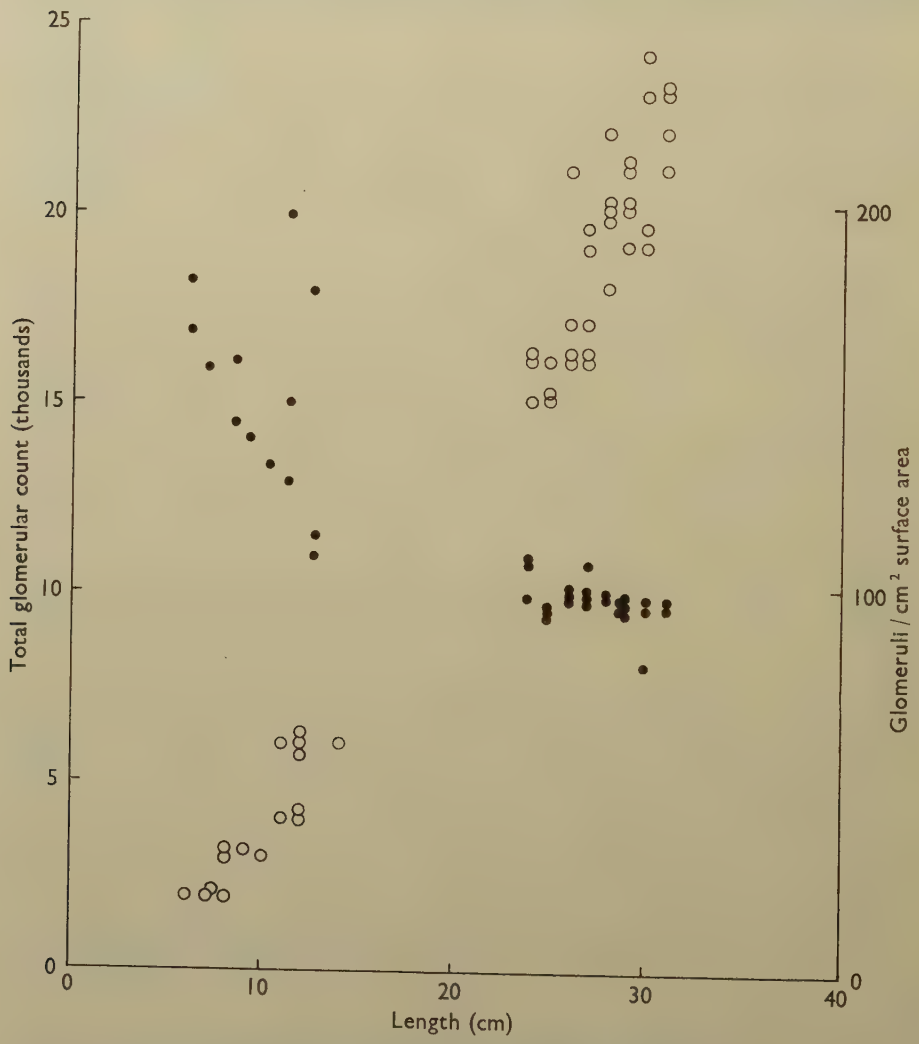

Fig. 7. Number of glomeruli in the kidney of herring of different length. ○, Total glomerular count; ●, glomeruli/cm² of surface area.

abundance on the pseudobranch (reported by Parry, Holliday & Blaxter, 1959, who demonstrated this in sea trout, *Salmo trutta*, and saithe, *Gadus virens*, as well as herring).

A continuation of this work on the pseudobranch (Parry & Holliday, 1960) showed that these acidophil cells seem to have another function and therefore no further details of this study in herring will be given here.

DISCUSSION

Salinity tolerance and functions of the kidney

One of the most intriguing aspects of the herring's biology is its ability to withstand low salinities (down to 6‰) for long periods of time, even after direct transfer from sea water. This tolerance is characteristic of both juvenile and adult fish, and our investigations on the larvae show an even wider range of tolerance. It is mainly in the juvenile stages that herring frequent regions which are truly estuarine, but the tolerance of a wide range of salinity is evidently not lost in the adult stages. The ability to withstand these salinity changes is undoubtedly related to the structure of the herring kidney. It possesses sufficient of the characters of the freshwater fish kidney (e.g. high glomerular count) to enable it to cope with water excretion in waters of reduced salinity, but not enough (e.g. no distal tubule) to allow the fish to penetrate into completely fresh water. This suggests that while the kidney is able to excrete excess water when necessary, it is not able to conserve salts, the latter being a reflexion of the normal marine habitat of the fish. It is the absence of the distal tubule that most probably precludes the salt conservation necessary for survival in fresh water.

The Malpighian bodies appear to retain the same structure and blood supply in water above and below 15·8‰—the salinity isotonic with the blood. However, when in water of greater salinity than this, it might be expected that water excretion in the urine would be at a minimal level, the emphasis being on water conservation. The excretion of more than minimal quantities of water must start to take place when the salinity of the water falls below 15·8‰ and the osmotic gradient is into the fish.

It is interesting to note that the number of glomeruli per unit surface area of body is highest in the 0-group fish. This may have a functional significance. It is at this stage that the fish most commonly enter estuarine waters where increased excretion of water is required and passage of water into the fish depends on the area of permeable surface.

The importance of damage to the skin for survival

In a previous publication (Parrish, Blaxter & Holliday, 1958) it was suggested that the high mortality of captured herring kept in sea water was the result of osmotic loss of water through the damaged epidermis, the process of

capture inevitably leading to a loss of scales. It was shown that this mortality could be considerably reduced by establishing the herring in water of reduced salinity, approximately isotonic with the blood, and that in many cases fish that were distressed in sea water would recover rapidly in one-third sea water.

The value of returning badly scaled herring (in particular trawl-caught) to the sea in the hope of their survival, as in many tagging experiments, is doubtful. The chances of dehydration mortality are very high. It would appear that at least 50% of the scales should be intact before the fish can commence any degree of control of water. If tagged fish could be returned to water of less than 15·8‰ for even a few hours before release into the sea it might increase the chances of survival of the less severely damaged fish by giving time for the adjustment of factors such as skin permeability (by mucus production) and urine production resulting from handling diuresis.

Evolution of the herring

The environmental origin of the teleosts is at the moment uncertain. Opposing views are held by Romer & Grove (1935), Romer (1955) and Robertson (1957). It is interesting to consider where the herring fits into this general picture.

In its immature stages the Atlantic herring is very often an estuarine fish, while in its adult stages, though usually remaining in the open sea, it still retains the ability to withstand estuarine conditions. During the spawning period it may occasionally move inshore to spawn (see Ford, 1929, and Brandhorst, 1959). However, the Baltic herring live in low salinities and the Pacific herring (*Clupea pallasii*) characteristically spawns much closer inshore.

The Atlantic herring, in its glomerular count and development of the egg on the sea bed, retains many of the characteristics of a freshwater fish. There are, indeed, many closely related species, such as the shads, which spend long periods in fresh water. It also retains in both the larval and adult form many features of a primitive teleost, and Kyle (1926) gives a relatively recent (Cretaceous) date for its colonization of the sea. On the other hand, the herring has many marine characteristics, including the lack of a distal tubule in the kidney, an oligolecithal egg and it needs only a short time between fertilization and hatching.

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SUMMARY

The salinity tolerance of herring 9–*ca* 24 cm in length was found to lie between 6‰ and 40–45‰.

Determinations of changes in weight and blood concentration (by measurement of the freezing-point), when herring were transferred from one salinity to another, demonstrated that extensive changes occurred in the blood. Under these conditions the herring experienced and survived blood concentrations equivalent to salinites of 13–22·5‰. A recovery to near normal ($\Delta 0{\cdot}95 \equiv 15{\cdot}8$‰) took place in all the salinities within the tolerance range.

Badly descaled herring in sea water showed large increases in blood concentration before death.

A study of the kidney of the herring indicated that the ability to withstand the low salinities for long periods probably rested in the high glomerular count of the kidney.

The importance of damage to the skin for survival is discussed in relation to tagging experiments.

The results are also discussed in relation to the evolution of the herring.

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THE LONG ROUGH DABS OF THE CLYDE SEA AREA

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Studies of the natural history of the long rough dab have been carried out at Millport over a number of years (Bagenal, 1955*a*, *b*, 1957*a*, *b*), and this paper describes the characters of the population in the Clyde Sea area.

The long rough dab was first described by Müller in 1776 and named *Pleuronectes linguatula*, but this name was preoccupied by *P. linguatula* Linnaeus, 1758, which is now called *Eucitharus linguatula* (L.). In 1780 Fabricius described a Greenland specimen, which he called *Pleuronectes platessoides* and which is in the Zoological Museum, Copenhagen, while Bloch in 1787 described a long rough dab caught off Heligoland which he named *P. limandoides*. The generic name *Hippoglossoides* was first used by Gottsche in 1835, and this must take precedence over *Drepanopsetta* used by Gill in 1862. The full synonymy is given by Norman (1934). The species is very variable, but nevertheless may be divided into an American and European subspecies.

The American race *Hippoglossoides platessoides platessoides* (Fabricius) is found on both sides of Greenland, as far north as Upernavik on the west coast (Jensen, 1925) and on the east coast in Denmark Strait and north to 72° 25′ N. (Jensen, 1904). It is found from Lake Melville and Hamilton Inlet (Frost, 1938; Backus, 1951) on the Labrador coast southwards into the Gulf of St Lawrence, round Newfoundland, on the Grand Banks (Templeman & Andrews, 1956) and it is numerous as far south-west as Cape Cod. A few have been caught in the Woods Hole region as far south as Narragansett Bay, though the most south-westerly record is of one caught off Montauk Point, N.Y. (Bigelow & Schroeder, 1953).

The European race *H. p. limandoides* (Bloch) is found all round Iceland (Saemundsson, 1949), Spitzbergen, Bear Island and the Faroes. It is found in the Barents Sea, and from the Murmansk Coast, round Norway and into the Baltic (Meyer, 1935; Krüger, 1942). In the North Sea it has been recorded as far south as Flamborough on the west side, and the Terschelling Light Vessel on the Dutch coast, and round the Dogger Bank as far south as the Silver Pit and Markham's Hole (Marine Biological Association, 1909, 1912). The long rough dab is found all round Scotland, the Lousy Bank (Pawsey, Atkinson & Davis, 1920), and into the Irish Sea as far as Dublin (Holt, 1910), though it is not common off the Isle of Man (Moore, 1937). It is found all down the west coast of Ireland to the Skelligs (Holt, 1892). The long rough

dab used to extend as far south as the English Channel (Yarrell, 1836, pp. 224–6; Day, 1880–84, pp. 9–10) but is not now recorded at Plymouth (Marine Biological Association, 1957). It was not recorded from the Bristol Channel by Lloyd (1941, 1942), but has been caught there commercially (Ministry of Agriculture and Fisheries, 1954, 1955).

The biology of the American race, which is called the American plaice, has been described by Huntsman (1918) and Bigelow & Schroeder (1953), while Templeman & Andrews (1956) have discussed the origin of the 'jellied' condition sometimes found in the flesh of specimens from the Grand Banks.

Apart from my work on the Clyde population (Bagenal, 1955*a*, *b*, 1957*a*, *b*), the biology of the European race has been considered at Iceland by Saemundsson (1908, 1925, 1927) in the Barents Sea by Essipow & Slastnikow (1932), Komerova (1939) and Milinsky (1944), while Krüger (1942) and Meyer (1935) have given details of the long rough dabs that are found in the Baltic.

A description of both subspecies is given by Norman (1934), whose data are summarized in Table 1.

TABLE 1. SUBSPECIFIC CHARACTERS OF *HIPPOGLOSSOIDES PLATESSOIDES*

Character	*H. p. platessoides* (western)	*H. p. limandoides* (eastern)
Depth of body: length of body	2·13–2·67	2·40–3·00
Maxillary: head length	2·67–3·00	2·25–2·60
Lower jaw: head length	2·10–2·20	1·83–2·00
Scales in lateral line	90–97	85–92
Scales between lateral line and middle of back	41–43	25–40
Dorsal fin rays	(80) 86–94	(76) 78–98 (101)
Anal fin rays	64–73 (75)	60–79

THE CLYDE SEA AREA POPULATION

Measurements from samples of long rough dabs caught in the main channel of the Firth of Clyde between the islands of Great Cumbrae and Bute are summarized in Table 2. All the measurements were made on fresh unpreserved fish with a pair of dividers and estimated to the nearest millimetre, and are the same as those used by Norman (1934). The length of the body was measured from the tip of the lower jaw to the base of the caudal fin which was taken as the edge of the hypural bones in the mid-line. In growth rate studies it is usually more convenient to measure the total length from the tip of the lower jaw to the end of the longest caudal fin ray, although this is less accurate in some specimens because of abrasion of the tail. Details of the ratio of the length of body to total length are given in Table 3. The depth of body was measured across the greatest depth of the fish excluding the marginal fins. This measurement is what in flatfishes is generally, though inaccurately, called the width. The length of the upper jaw is measured from the extremity of the snout and includes the maxillary and premaxillary. This, and the

length of the lower jaw are measured on the right (ocular) side. The length of the head is measured from the tip of the snout to the hind edge of the bony operculum.

TABLE 2

Character	No. examined	Mean	Standard deviation	Range
Depth of body: length of body	50	2·70	0·19	2·48–3·29
Upper jaw: head length	35	2·54	0·19	2·33–3·42
Lower jaw: head length	35	2·00	0·02	1·88–2·19
Head length: length of body	50	3·78	0·17	3·48–4·23
Scales in lateral line	42	88·86	1·93	85–92
Scales between lateral line and middle of back	42	28·86	1·86	25–32
Dorsal fin rays	136	82·65	3·10	75–90
Anal fin rays	136	65·77	2·32	61–72

TABLE 3

	No. examined	Mean ratio	Standard deviation	Range
Ratio of body length to total length	42	1·21	0·019	1·16–1·25

Scale counts

The scales in the lateral line were counted just above the line itself from upper angle of the operculum to the base of the caudal fin, while the scales between the lateral line and middle of the back were counted in an oblique row. It was found that these scale counts were not easy to make and had to be attempted several times before a definite decision was reached. The dorsal and anal fin rays were easily and more accurately counted, and are considered in greater detail below.

The scale counts in Table 2 correspond very well with those given by Norman, but the size of the jaws of Clyde specimens would seem to be larger than was generally found by Norman, though 33 of his 76 whole specimens examined came from the Clyde Area. For example, the mean lower jaw: head length ratio is the same as the upper limit for the range given by Norman. However as Pietschmann (1932) found with Greenland specimens, the body proportions change with age. The only reliable diagnostic racial characters are the number of scales in the lateral line and the number between the lateral line and the middle of the back.

Fin ray counts

As was first mentioned by Collett (1880), the number of dorsal and anal fin rays can be shown to increase with increasing latitude and this is also associated with increased number of vertebrae, scales and pores in the lateral line. This point was followed up by Norman (1934) who gives the ranges of fin ray counts, together with those of scales, for a number of specimens from various localities. Table 4 has been prepared from Collett and Norman, together with data from the Clyde and Baltic, and illustrates

the relation of dorsal fin ray numbers and degrees of latitude. It can be seen that the association is quite definite although there is a considerable overlap and the number of specimens is unfortunately low. Townsend (1937), with the Pacific species, *H. elassodon* Jordan & Gilbert, noted a similar phenomenon: 'The regular increase in number of meristic characters towards the west and north noted in the samples of this species indicates that the differentiation of racial characters is a regular process along our coast.'

TABLE 4. THE NUMBER OF DORSAL FIN RAYS OF LONG ROUGH DABS FROM DIFFERENT LOCALITIES

Locality	Authority	Approximate latitude	No. examined	Dorsal fin rays No.	Dorsal fin rays Range
Spitzbergen	Collett	77°	15	90·8	84–101
	Norman	.	3	.	85–98
Bear Island	Collett	74° 30′	1	87	.
	Norman	.	1	85	.
E. Finmark	Collett	70° 30′	8	92·4	89–97
Tromsø	Collett	69° 40′	3	87·0	85–91
Iceland	Norman	*c.* 65°	1	95	.
Bergen	Norman	60° 23′	5	.	78–81
Oslo Fiord	Collett	59° 30′	7	83·1	79–87
British Isles	Norman	.	35	.	77–88
Millport	Bagenal	55° 47′	136	82·7	75–90
Baltic	Meyer	54° 30′	231	82·1	75–89

The relation of dorsal and anal fin ray numbers in the Clyde samples (Table 5) was investigated and the correlation coefficient of $r = 0{\cdot}65$ is highly significant. The correlation coefficients found by Townsend for *H. elassodon* from Puget Sound was $r = 0{\cdot}910$ and from Alaska $r = 0{\cdot}768$. The fin ray numbers do not however have any relation to the total fish length (correlation coefficient $= 0{\cdot}108$ is not significant).

Vertebral counts

In the first instance counts were made of the total number of vertebrae, not including the urostyle, of 51 long rough dabs. Details are given in Table 6. Following the advice of Ford (1937) the total was split into two regions, abdominal and caudal. The results of these counts are given in Table 7. The fish with 12 abdominal vertebrae have significantly more in the caudal region than those with 13 abdominal vertebrae.

Vertebrae showing signs of fusion of two separate segments were noted in only one case, in which the 3rd and 4th abdominal vertebrae were fused and this occurred in an individual that, if the fused ones are counted as a single unit, had 12 abdominal vertebrae. More commonly the only indication of fusion was a doubling of the number of neural spines on the last vertebra. This occurred in six of the fifty-one examined, once each where the total

was 42 and 45, and four times where the total number of vertebrae was 43. In only two of these six cases was there a doubling of the haemal spines as well, and there were no cases of extra haemal spines alone.

TABLE 5. RELATION OF ANAL AND DORSAL FIN RAY NUMBERS

	Anal Fin Rays												
	61	62	63	64	65	66	67	68	69	70	71	72	Total
90	.	.	.	.	.	.	.	2	1	.	.	.	3
89	.	.	.	.	.	.	1	.	.	1	.	1	3
88	.	.	.	.	.	2	1	.	.	1	.	.	4
87	.	.	.	.	.	.	.	2	.	2	.	2	6
86	.	.	.	.	.	1	1	4	1	.	.	.	7
85	.	.	.	.	1	1	2	2	3	.	.	.	9
84	.	.	1	5	1	6	4	3	2	.	.	.	22
83	.	.	1	2	5	3	1	2	.	.	.	.	14
82	.	1	3	3	3	3	2	1	1	.	.	.	17
81	1	.	4	5	5	2	2	2	.	.	.	.	21
80	1	.	1	3	.	2	2	1	.	.	.	.	10
79	1	.	3	2	1	1	.	.	.	.	.	.	8
78	.	.	1	3	2	.	.	.	.	.	.	.	6
77	.	2	1	.	.	.	.	.	.	.	.	.	3
76	.	.	.	1	.	1	.	.	.	.	.	.	2
75	1	.	.	.	.	.	.	.	.	.	.	.	1
Total	4	3	15	24	18	22	16	19	8	4	.	3	136

TABLE 6. THE DISTRIBUTION OF THE TOTAL NUMBER OF VERTEBRAE

Total no.	Frequency
45	4
44	21
43	23
42	3
Total	51
Mean	43·5

TABLE 7. THE DISTRIBUTION OF ABDOMINAL AND CAUDAL VERTEBRAE

Caudal vertebrae	Abdominal vertebrae		Total	Mean
	12	13		
33	1	.	1	12
32	8	3	11	12·27
31	13	13	26	12·50
30	2	10	12	12·83
29	.	1	1	13·0
Total	24	27	51	.
Mean	31·5	30·7	.	.

The relation of vertebrae and fin rays

Typically each vertebra has a haemal and, in the caudal region, a neural spine and with each spine are associated two fin rays. The relation of vertebrae and dorsal fin rays was examined in fifty-one fish and the details are given in Table 8 where it can be seen that those fish with more vertebrae tend to have more fin rays, but that there is a large amount of variation.

TABLE 8. THE RELATION OF VERTEBRAE AND DORSAL FIN RAYS

Dorsal fin rays	Vertebrae 42	43	44	45
77	1	.	.	.
78	.	4	.	.
79	.	2	1	.
80	.	3	2	.
81	2	3	4	2
82	.	2	3	.
83	.	3	3	.
84	.	1	2	.
85	.	3	.	.
86	.	1	1	.
87	.	.	2	1
88	.	1	2	.
89	.	.	.	1
90	.	.	1	.
Mean	80	81·7	83·4	85

Reversed forms

In the studies of growth, breeding and fecundity that have been carried out at Millport with long rough dabs from the Clyde area, 2357 specimens were examined in detail, and only one of these (a female of 18 cm) was a reversed form lying on its right side instead of its left. Although many thousands of other long rough dabs have been caught and some superficially examined, we have no other records of sinistral individuals. The only other specimens I know of is one recorded by Meyer (1934) and three recorded by Krüger (1942), all from the Baltic.

Commercial importance

The long rough dab is of little importance commercially in the Clyde Area. It is not considered in the Scottish Sea Fisheries Statistical Tables (Scottish Home Department), though since 1951 it has been recorded in the tables relating to England and Wales (Ministry of Agriculture and Fisheries, 1951–58) which are summarized in Table 9. It can be seen that both subspecies are landed and that most are brought from northern waters where the fish grow considerably bigger (Saemundsson, 1925; Essipow & Slastnikow, 1932; Milinsky, 1944; Bagenal, 1955*a*). In the southern part of its range the long rough dab is discarded as useless. While the British landings appear to be declining, in Canada the American plaice is growing in commercial importance (Templeman & Andrews, 1956).

SUMMARY

The biometry of the Clyde population of long rough dabs has been studied and compared with the descriptions of the European race given by Norman in his monograph of the flat-fishes. The data consist of various body measurements

together with the results of scale counts, fin ray counts and vertebral counts. The fin ray numbers fit into a pattern of increasing number with high latitudes when compared with other published data. There is a correlation between the number of anal and dorsal fin rays, and between these and the number of vertebrae. The variations that were found in the vertebrae of fifty-one fish are described. Only one sinistral long rough dab has been found out of 2357 specimens examined. From the British fishing statistics it is shown that the long rough dab is of little, and declining, commercial importance. In Canada, however, the American subspecies is becoming more important commercially.

TABLE 9. LANDINGS IN CWTS. OF LONG ROUGH DABS AT BRITISH PORTS BY BRITISH VESSELS FROM VARIOUS FISHING REGIONS FROM 1951 TO 1958.

	1951	1952	1953	1954	1955	1956	1957	1958
Barents Sea	1499	1855	572	354	321	35	149	258
Bear Island and Spitzbergen	798	156	109	90	40	41	128	625
Norwegian Coast	.	145	12	16	.	.	2	.
Iceland	176	121	45	5	16	6	31	29
Faroes	6	.	.	.	.	.	.	.
North Sea	6	2	125	7	5	3	.	.
West of Scotland	1	.	.	.	.	.	.	.
Rockall	.	.	.	.	.	.	.	.
West of Ireland	.	.	.	.	1	.	.	.
Irish Sea	.	.	9	.	8	.	.	.
Bristol Channel	.	.	4	1	21	2	.	.
South of Ireland	.	.	.	.	.	.	.	.
English Channel	.	.	.	.	.	.	.	.
Labrador	.	493	122	.	.	.	.	.
West Greenland	.	6	56	5	.	.	31	42
Newfoundland Grand Banks	.	.	101	2	.	.	749	.
Total	2486	2778	1155	480	412	87	1090	954

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THE LARVAL STAGES OF *BALANUS BALANUS* (L.) DA COSTA

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(Text-figs. 1-4)

Balanus balanus is a common boreo-arctic cirripede, occurring in relatively shallow waters on both sides of the North Atlantic and in North Pacific coastal waters. Its general biology has been described (Barnes & Barnes, 1954; Crisp, 1954), and although the larvae were recognized by one of us (H.B.) and separated from plankton samples several years ago, they have never been described. Throughout the whole area of the distribution of the species there appears to be only a single annual breeding period and the larvae are released during the early spring at about the same time as those of *B. balanoides* and much *B. crenatus* together with *Verruca stroemia*. Locally the larvae of *Balanus balanus* may be abundant—indeed often equal in quantity to those of *B. crenatus* with which they have probably been confused.

The larvae described below were, with the exception of the cyprid, obtained in culture. Stage I nauplii taken from ripe egg masses were transferred to the individual compartments of plastic boxes containing sea water with the addition of diatom cultures: the method has been developed and found satisfactory by Costlow & Bookhout (1957, 1958). *Skeletonema costatum* alone and mixed with *Biddulphia regia*, *Ditylum brightwellii*, and *Phaeodactylum cornutum* were used as food for the developing larvae. Some 10–20 larvae were kept in each compartment and as the stages developed they were picked out and preserved in either alcohol or 5 % formalin. The drawings were made from the preserved material. The cultures were kept cool, 6–10° C, and some were shaken. Only a few rather misshapen cyprids were obtained, and this stage has, therefore, been drawn from planktonic material—recognized by following it to the young adult. Since in natural plankton samples larvae of *Balanus balanoides* and *B. crenatus* (both of which have been described) will often accompany *B. balanus*, reference will often be made throughout the description to points of similarity or contrast.

General features of the larvae

Six naupliar and a cyprid stage were found; outline drawings are given in Fig. 1 and some dimensions in Table 1. The total length was measured from

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the mid-point of the frontal margin to the tip of the caudal spine, the carapace length from the mid-point of the frontal margin to the mid-point of the posterior border of the carapace, and the carapace width was taken as the greatest width behind the fronto-lateral horns.

In size, as in many other characters, the larvae are similar to *B. balanoides* and *B. crenatus*, being less bulky than the former yet hardly so delicate looking as the latter. The general outline—elongated body, laterally directed and

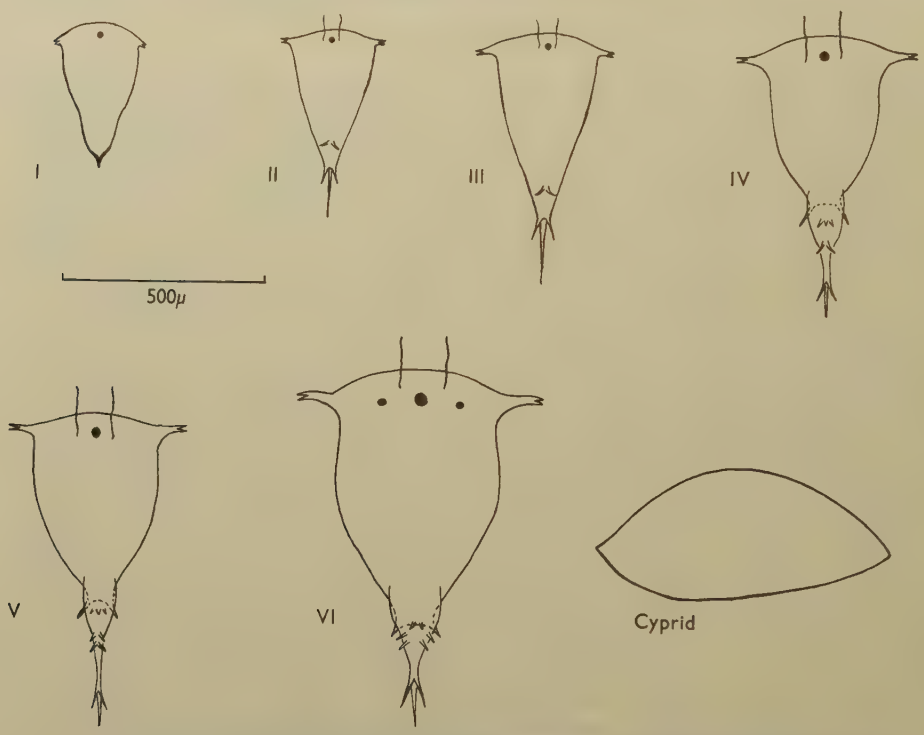

Fig. 1. Outline drawings of the six naupliar stages and the cyprid stage of *Balanus balanus* showing the shape and the more important spinous processes.

TABLE 1. SIZE OF THE LARVAL STAGES OF *BALANUS BALANUS*

(Larvae taken from laboratory cultures.)

Stage	Total length (mm)	Carapace Length (mm)	Carapace Width (mm)
I	0·37	—	0·21
II	0·51	—	0·25
III	0·62	—	0·26
IV	0·66	0·40	0·31
V	0·76	0·51	0·36
VI	0·92	0·61	0·42
Cyprid	0·81	(planktonic material)	

prominent fronto-lateral horns—closely resembles that of *B. crenatus*. Stage I has a more or less pear-shaped body and the fronto-lateral horns are at first closely appressed to the body; this stage rapidly passes into stage II and is rarely taken in plankton collections. The caudal process in stage I is relatively short; there are no frontal filaments. A median eye is present in this and all subsequent stages.

Stage II has moderately prominent fronto-lateral horns and the body in outline is even more triangular than that of *B. crenatus* and more so than that of *B. balanoides*. Frontal filaments are present in this and all subsequent stages. The length of the abdominal process is about half that of the caudal spine so that the resemblance here is to *B. balanoides* (Bassindale, 1936; Pyefinch, 1948). The abdominal process bears a single pair of spines and forms at its posterior extremity a well-defined furca, whose slender straight rami diverge at an acute angle.

Stage III, although somewhat larger, closely resembles stage II in outline. The somewhat triangular outline is still maintained, the fronto-lateral horns project well to the side, and the caudal spine is still much longer than the abdominal process. As in many other species, the antennule has a single pre-axial seta not present in stages I and II.

At stage IV, the posterior border of the carapace becomes clearly defined and laterally bears a pair of spines which are moderately long, tapering, and in comparison with some species set rather close together. The outline is less triangular, becoming in this and the two subsequent naupliar stages much more rounded, with the lateral margins of the carapace bowed outwards. Stage V has a much enlarged abdominal process whose length is now almost equal to that of the caudal spine. Stage VI has the cirriform appendages, now fully segmented, clearly visible beneath the exoskeleton of the abdominal process which is much enlarged and subequal in length to the caudal spine. Paired lateral eyes are present in the older individuals of the stage, situated at either side of the median eye; in larvae from cultures the paired eyes gradually develop pigment but in most planktonic material examined the pigment is dense.

The cyprid is much paler than *B. balanoides* cyprids and is a little more elongated as is that of *B. crenatus*.

The carapace

A distinct border to the carapace, bearing laterally placed spines, first appears in stage IV and much evidence now suggests that this may be true of all balanid nauplii (Jones & Crisp, 1954; Costlow & Bookhout, 1957, 1958; Barnes & Barnes, 1959*a*, *b*). Pyefinch's (1948*a*, *b*) description of stage III *B. balanoides* and *B. crenatus* in which he indicates a posterior border with spines appears to be erroneous. Norris & Crisp (1953) considered that Pyefinch overlooked the real stage III in *B. balanoides* and described stage IV

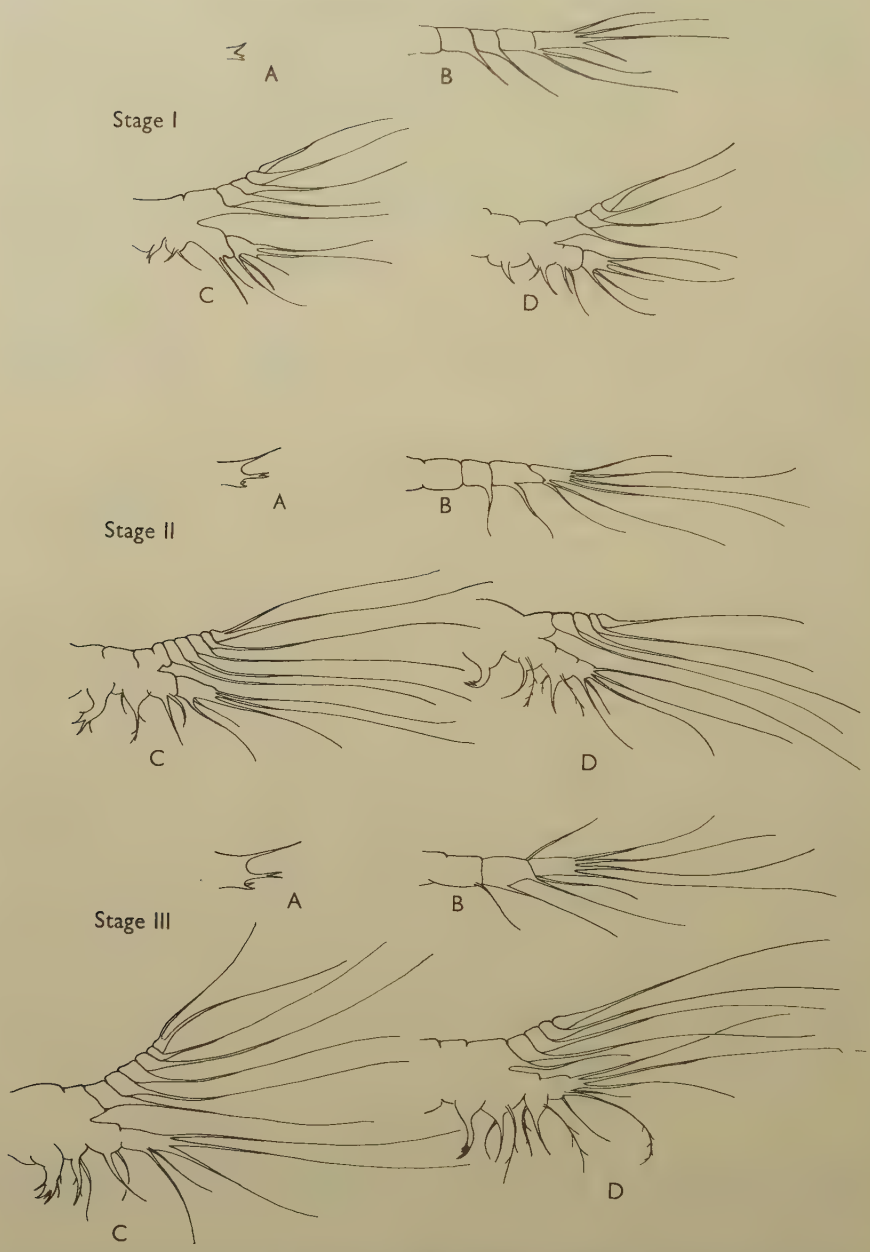

Fig. 2. Stages I, II and III larvae of *Balanus balanus*: (A) lateral view of abdominal and caudal processes, (B) antennule, (C) antenna, (D) mandible. For scales see Fig. 3.

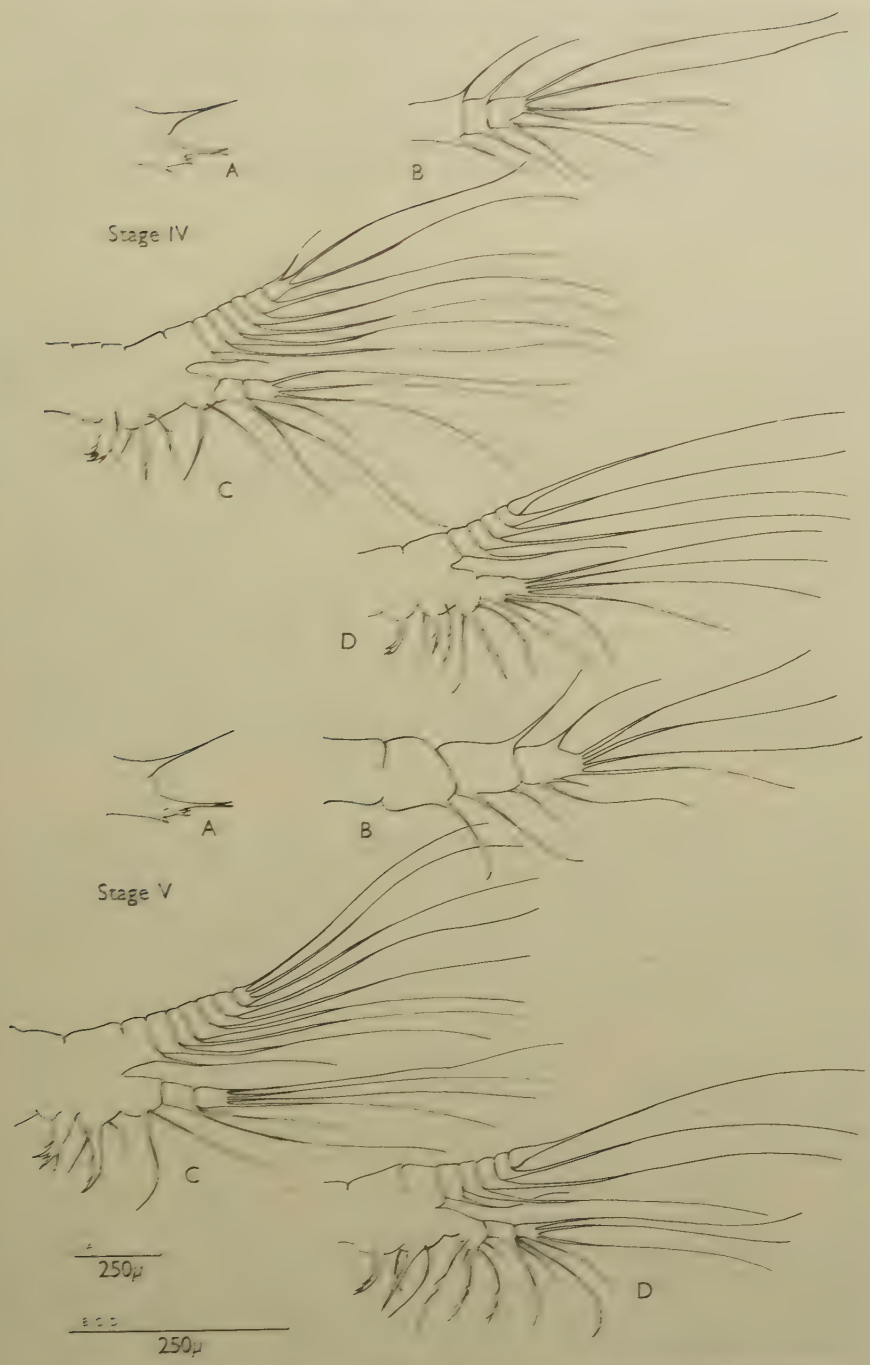

Fig. 3. Stages IV and V larvae of *Balanus balanus*: (A), (B), (C) and (D) as in Fig. 2.

as stage III. Since Pyefinch did not recognize *B. balanus* in his collections—and there must have been a very considerable number present—it seems more likely that stage IV *B. balanus* was described as stage III *B. balanoides*. He does not give a setation formula but a comparison of his sizes with those in Table 1 supports this suggestion. Bassindale's (1936) figures, although showing a distinct border in stage III *B. balanoides*, do not show carapace spines until stage IV is reached.

Abdominal process and caudal spine

Apart from variations in their relative lengths, the form and character of these are similar in all balanids. In the earlier stages of *B. balanus* the abdominal process is only half the length of the caudal spine so that in this respect the larvae are similar to those of *B. crenatus*. In stages V and VI, however, the processes are subequal. The spines found on the abdominal process appear to form a uniform pattern in many species that have so far been examined. Stages II and III have a single pair of spines laterally placed. Stage IV has a further pair of spines, farther to the anterior and more ventrally placed, and between these a single median spine. Yet a further pair of spines is added in stage V; this stage has, therefore, a total of seven spines on the process. The median spine of the second series is lost in stage VI. The arrangement and sequence is common to many species (Jones & Crisp, 1954; Knight-Jones & Waugh, 1949; Sandison, 1954; Barnes & Barnes, 1959*a*, *b*), but has not always been described in others where the spines have not been referred to or drawn.

Setation of the appendages

Paired antennules, antennae and mandibles are present in all stages. Stages II–VI have slender frontal filaments that arise on either side of the median eye. Figs. 2–4 show the setation of the appendages and their form, adopting the now conventional notation of Bassindale (1936), is set out in Table 2. As in many other species a pre-axial seta on the antennule is first added in stage III and a further one is found in stage IV. Unlike many other species—but not all—stage V has only two pre-axial setae on the antennule; most species have three at this stage. The gnathobase of the antenna is covered with a complex series of spines but that of the mandible is rather more simple. Only a limited number of individuals from the cultures were examined, but in these no evidence of variability in setation was obtained; setae vary in length and position slightly from one individual to another, but Table 2 applies to all the specimens examined. The variations described by Norris, Jones, Lovegrove & Crisp (1951) and which they think may be particularly common in cold-water species were not found: they may, nevertheless, be present in natural populations that have developed under less constant conditions.

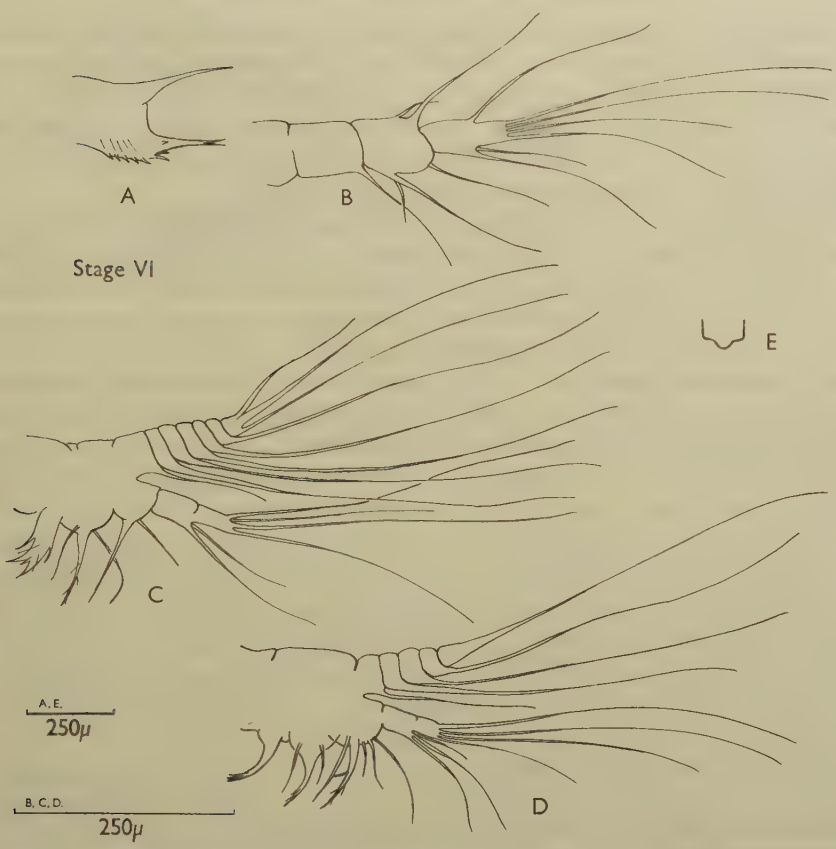

Fig. 4. Stage VI larva of *Balanus balanus*: (A), (B), (C) and (D) as in Fig. 2; (E) labrum.

TABLE 2. SETATION FORMULAE FOR THE NAUPLII OF *BALANUS BALANUS*

(All notations according to those given by Bassindale.)

Stage	Antennula	Antenna	Mandible
I	0.4.2.1.1.	0.1.4.–0.3.2.2.2.G.	0.1.3.–0.3.2.2.2.G.
II	0.4.2.1.1.	0.2.4.–0.3.2.2.2.G.	0.1.3.–0.3.2.3.2.G.
III	1.4.2.1.1.	0.2.5.–0.3.2.2.2.G.	0.1.4.–0.3.3.3.3.G.
IV	1.1.4.2.1.1.	0.3.6.–0.4.2.2.3.G.	0.1.4.–0.4.3.3.3.G.
V	1.1.4.2.1.1.1.	0.3.6.–0.4.2.2.3.G.	0.1.4.–0.4.3.5.3.G.
VI	1.1.1.4.2.1.2.1.	0.3.6.–0.4.2.2.3.G.	0.1.4.–0.4.3.5.3.G.

DISCUSSION

Although it has sometimes been said that the setation of the appendages is of little use in specific recognition it is becoming increasingly evident, as more larvae are described, that if all the setae are considered then the setation could possibly be used in specific recognition. While many species have one or more

limbs identical, so far as is known, no species has identical setation on all the limbs of all the stages. From the point of view of larval recognition in general plankton work when stages must be separated, examination of the total setation is of little practical use. It is usually possible to rely on more gross features, taken in conjunction with the known breeding seasons of the species present in any particular region, for rapid recognition.

B. balanus is boreo-arctic in distribution, breeding in the spring. Therefore, both in Europe and on the American Atlantic coast the problem of recognition is essentially that of distinguishing its larvae from those of *B. balanoides and B. crenatus*. (The small size and distinctive appearance of *Verruca stroemia* larvae which may be present in European samples of spring plankton make it easily recognized.) On the Pacific coast of North America a distinction will also have to be made with two further species, *Balanus cariosus* and *B. glandula*. It may be noted that the larvae of *B. hameri* have not yet been described and this species also releases its larvae in the spring. However, it is much less common, and tends to be found in deeper water.

The difficulties of separation from *B. balanoides* and *B. crenatus* are real; *B. balanus* was not recognized by Pyefinch (1948*a*) in his samples, although it is abundant in the Clyde Sea Area in the spring. The larvae of *B. balanus* are more delicate than those of *B. balanoides* but resemble this species in the relative lengths of the abdominal process and caudal spine in stages II–IV. *B. crenatus*, very similar in size, is rather less triangular particularly in the early stages than is the case with *B. balanus*.

It would be a relatively simple matter to make an identification key for all the common British balanomorph barnacles. The frequent wide separation in distribution and quite distinct breeding periods makes this unnecessary; it is more profitable to have keys for distinct areas and seasons. *B. balanoides*, *B. balanus* and *B. crenatus* occur together over a wide range in Northern Europe with their larvae present together in the plankton.

The following key enables the three species to be separated:

1. Posterior border of carapace not defined: no carapace spines. **3**
2. Posterior border of carapace well defined: carapace spines. **6**
3. Abdominal process much shorter than caudal process. **5**
   Abdominal process subequal to caudal process. **4**
4. Single pre-axial seta on antennule. *B. crenatus*, stage III
   No pre-axial seta on antennule. *B. crenatus*, stage II
5. Larva roughly triangular in outline: frontal margin only moderately bowed out anteriorly. *B. balanus*, stages II and III
   Larva more globose: frontal margin strongly bowed. *B. balanoides*, stages II and III
   (Separate Stages II and III in 5 as for 4).

6. Larva with paired lateral and median eye: spines containing tips of cirriform appendages present on abdominal process. 7
   Larva with single median eye. 9
7. Delicate nauplius, somewhat elongate. 8
   Bulky nauplius with abdominal process somewhat shorter than caudal process: furca of former not diverging greatly. *B. balanoides*, stage VI
8. More delicate nauplius: abdominal process and caudal process subequal: general outline globose. *B. crenatus*, stage VI
   More delicate nauplius: abdominal process and caudal process subequal: general outline wine glass shape. *B. balanus*, stage VI
9. Larva with only five spines on abdominal process. 10
   Larva with total of seven spines on abdominal process. 12
10. Larva rounded with short abdominal process. *B. balanoides*, stage IV
    Larva less bulky, longer abdominal process. 11
11. Larva somewhat globose shaped. *B. crenatus*, stage IV
    Larva somewhat wine glass shaped. *B. balanus*, stage IV
12. Separate Stage V larvae of all species as in 10 and 11.

There has recently been much interest in the suitability and nutrient value of various kinds of food in the survival and development of crustacean larvae. The primary object of the present work was to obtain a series of larvae for description, but it is worth pointing out that, as with many cold water forms, survival particularly in the later stages was poor. Moyse (1960) has recently contrasted his extremely successful rearing of *Elminius modestus*—over 90% survival of the larvae to the cyprid stage—with the results of other work. However, in assessing the efficiency of a given food or the effectiveness of any culture technique caution should be exercised in comparing survival rates of different species if their times of development are much different. The results quoted for *E. modestus* were carried out at 22° C and only 6 days were required for the whole larval sequence. At its normal temperature of development and used in culture experiments the whole sequence for a cold water form could take 15–25 days. It would, therefore, be interesting to have for comparison the survival values obtained by Moyse for the cold water species which he raised by the same technique.

SUMMARY

Six naupliar and a cyprid stage for *B. balanus* are described and diagrams given for the general outlines and individual appendages. Sizes and setation formulae are given.

The naupliar stages resemble both *B. balanoides* and *B. crenatus*; attention is drawn to some distinguishing features and a key for the separation of the species is provided.

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VITAMIN B_{12} AND MARINE ECOLOGY: THE RESPONSE OF *MONOCHRYSIS LUTHERI*

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(Text-figs. 1–4)

I have suggested that the magnitude of the vitamin B_{12} requirement in algae, the amounts of vitamin recorded in the sea and the sizes of recorded plankton crops are such that there must normally be an abundance of this vitamin for any crops which might require it (Droop, 1957). The argument is summarized in Fig. 1. Among the criticisms offered by Daisley (1957) and Ford (1958) are:

(1) Vitamin B_{12} may occur in a 'bound' form unavailable to micro-organisms until autoclaved, in which case current bioassay techniques give too high an estimate.

(2) Agreement in the calculated magnitude of requirement (3 molecules per μ^3 of alga) between different algae may be a matter of chance, the sample being too small. Furthermore, greatly enhanced requirement occurs under conditions of temperature stress in at least one species (*Ochromonas malhamensis*) (Hutner *et al.*, 1957).

(3) In nature competition may be such that the dosage/rate rather than the dosage/yield relation is of paramount importance. Ford (1958) has shown that the rate of cell division in *Ochromonas* does indeed respond critically to levels of vitamin B_{12} concentration well above the limit of those measured in the sea.

The object of this paper is to present some dosage/rate curves for *Monochrysis* as they are very different from Ford's *Ochromonas* curves.

METHODS

Culture conditions

Monochrysis lutheri (Millport no. 60) is an obligate phototroph and makes use of no organic compound as a source of carbon. The culture medium, S 50 (Droop, 1958), is effectively a mineral one. Cultures were incubated with a light intensity of 2000 lux ('warm white' fluorescent lamps). 2000 lux appears to be near, but not quite at, the saturation intensity: 4000 lux is found to give a 10 % increase in the rate of cell division. Culture vessels were cotton-wool plugged 'Pyrex' test tubes holding 6 ml. of medium. No aeration or agitation was given. However, under these still conditions division rate was found to be independent of cell numbers until the latter reached two to four million cells per ml. Therefore, variations in both carbon dioxide content of the medium and mutual screening with increasing cell density were of

negligible proportions until this figure was reached. This limitation necessitated calculating division rates from measurements of early phases of cultures having cell densities ranging from 8 to 200 thousand cells per ml.

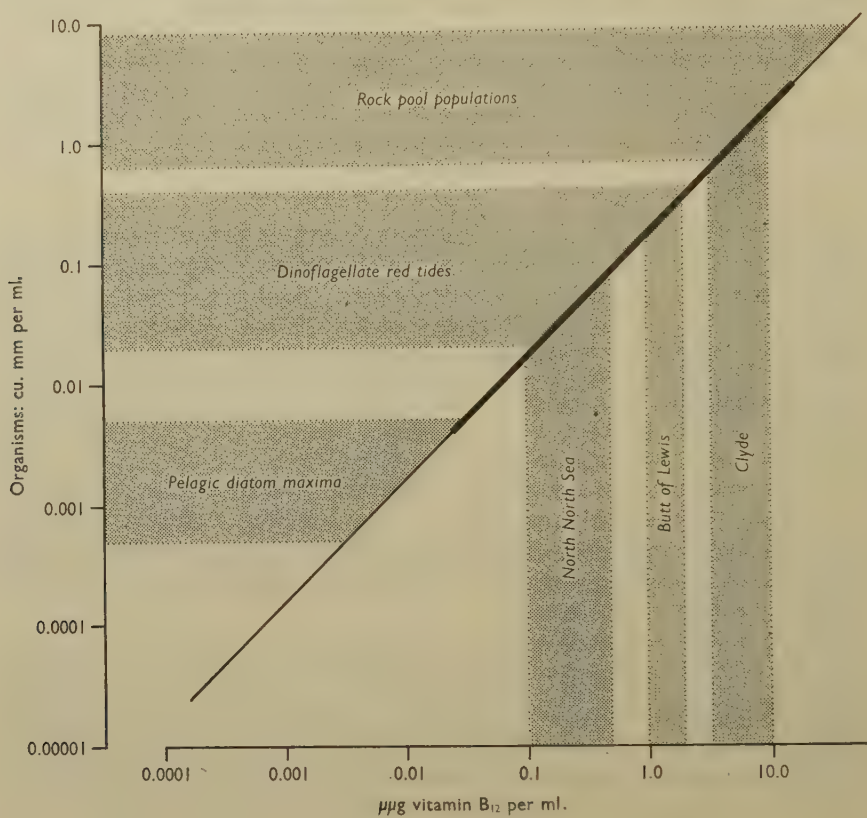

Fig. 1. Dosage/yield relation for *Monochrysis* and vitamin B_{12} on logarithmic co-ordinates. Heavy line, experimental; thin line, extrapolation; yield calculated as volume of living cells per ml. On the same scales are indicated for comparison: (1) the levels reached by some natural populations; (2) some measurements of vitamin B_{12} in the sea (data and references in Droop, 1957).

Preparation of inoculum

Use of early phases of cultures required carry-over of vitamin B_{12} to be reduced to an absolute minimum. I have not found it possible regularly to prepare cultures containing less than 0·1 $\mu\mu$g vitamin B_{12} per ml., but with due care in preparing glassware, media and inoculum this level can be achieved. Inoculum for experimental cultures was prepared as follows: mature stock cultures containing 100 $\mu\mu$g vitamin B_{12}/ml. were used to inoculate a 'vitamin-free' culture (ratio of inoculum to culture volume, 1:100). This, containing

about 1 $\mu\mu$g vitamin B_{12}/ml., would grow to a density of half a million cells per ml. before being arrested by vitamin B_{12} deficiency. It would then be diluted by a factor of 100 with vitamin-free medium and used to start the experimental cultures (again with an inoculum to culture ratio of 1 : 100). The initial density in the experimental cultures was reckoned to be of the order of 50–100 vitamin-limited cells per ml. It did, in fact, take a week before cells became sufficiently numerous (16 thousand per ml.) to be counted in a Fuchs Rosenthall haemocytometer.

Counts

In the earliest counts, when the whole of the ruled area of the Fuchs Rosenthall was required for a single estimation, at least three samples needed to be withdrawn and counted. In later counts sufficient statistical coverage could be obtained from a single sample. For still later counts it was necessary to resort to a Thoma haemocytometer.

Experiments

Three experiments were carried out, at 15°, 20° and 25° C, respectively. Each consisted of a series of unreplicated cultures with vitamin B_{12} dosage increasing geometrically from 0·01 to 100 $\mu\mu$g/ml.

RESULTS

Yield relation

Growth curves for the 15° experiment are shown in Fig. 2; the other two experiments were similar. The earliest counts revealed that all cultures were growing at a rate of approximately 1·1 divisions per day. Then, as the vitamin became exhausted in successive cultures, the growth curve of first one then another flattened off so that cell numbers finally came to give the familiar dosage/yield relation. After 27 days growth was virtually arrested in all but the two cultures with the highest vitamin B_{12} concentration. A plot of cell numbers against dosage on linear co-ordinates is shown in Fig. 3. The regression is seen to be linear, and is fitted by the equation

$$n = 0{\cdot}55 \times 10^6 (c + 0{\cdot}1).$$

Thus, each micromicrogram of vitamin B_{12} supported $0{\cdot}55 \times 10^6$ cells, which compares with values of 10^6, $0{\cdot}8 \times 10^6$ and $0{\cdot}5 \times 10^6$ obtained in previous experiments (Droop, 1957). The constant 0·1 indicates the size of the apparent carry-over, for it is the amount by which all values on the dosage scale must be increased for the regression to pass through the origin. An estimate of the actual, as opposed to the intended, vitamin content of a culture is given by the sum of the vitamin added intentionally and the apparent carry-over, and is referred to as 'corrected dosage'.

In the 25° experiment apparent carry-over was 0·4 $\mu\mu$g/ml. and in the 20° experiment it was 2·0 $\mu\mu$g/ml.

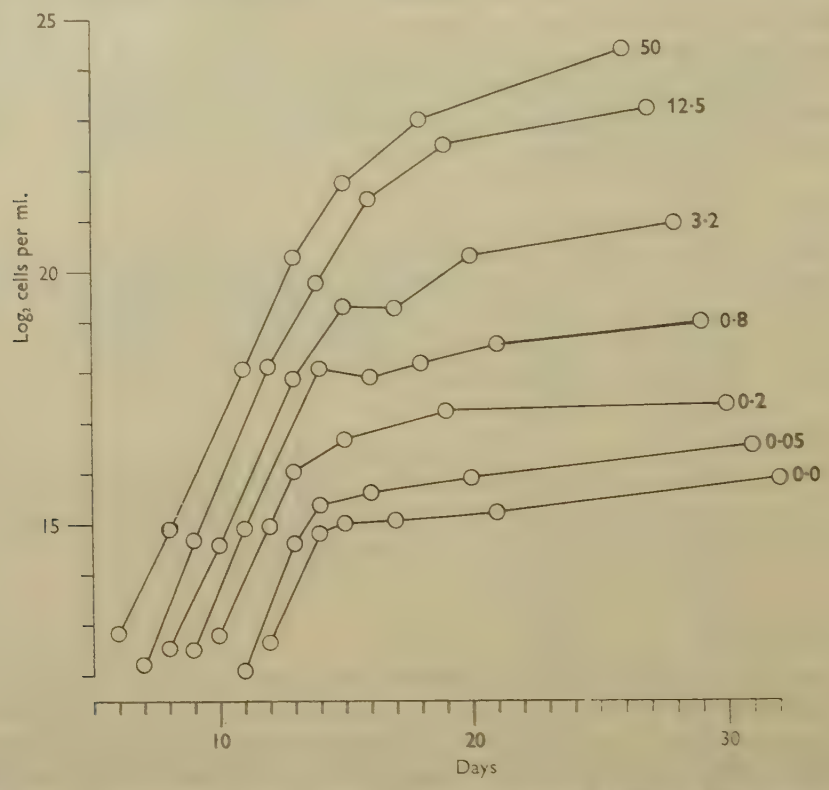

Fig. 2. 15° C experiment; growth curves. The vitamin dosage in $\mu\mu$g ml. (uncorrected) is indicated on each curve. For clarity successive curves are displaced on the time scale.

Rate of cell division

Monod (1942) expressed the relation between concentration of limiting nutrient (c) and relative growth rate (k) as

$$\frac{k}{k_{\text{max.}}} = \frac{c}{c+c_1}.$$

A nutrient is limiting only when c is not large compared to the constant c_1, for only then can k differ from $k_{\text{max.}}$. (c_1 has the dimensions of a concentration and is referred to as 'limiting concentration': when $c = c_1$, $k = \frac{1}{2}k_{\text{max.}}$.) The equation implies that, so long as cell numbers are sufficiently small as not to alter appreciably the concentration of the nutrient in question during the period of measurement, the relative growth rate will be constant over the period, even when the concentration is a limiting one.

The division rate during this initial logarithmic phase of constant nutrient concentrations was calculated for each culture by the normal method of regression analysis of the counts after their transformation to binary logarithms. These rates, with their 95 % fiducial limits, are entered in Fig. 4 against the corrected vitamin B_{12} dosage. No regular significant deviation from the mean value of 1·1 divisions per day is to be seen in the initial rates of any of the cultures in the 15° and 20° experiments, the conclusion being that at these temperatures vitamin B_{12} concentration between the limits 0·1 and 100 $\mu\mu$g/ml. is quite without limiting effect on the division rate and that the 'limiting concentration' (c_1) must be below 0·1 $\mu\mu$g/ml.

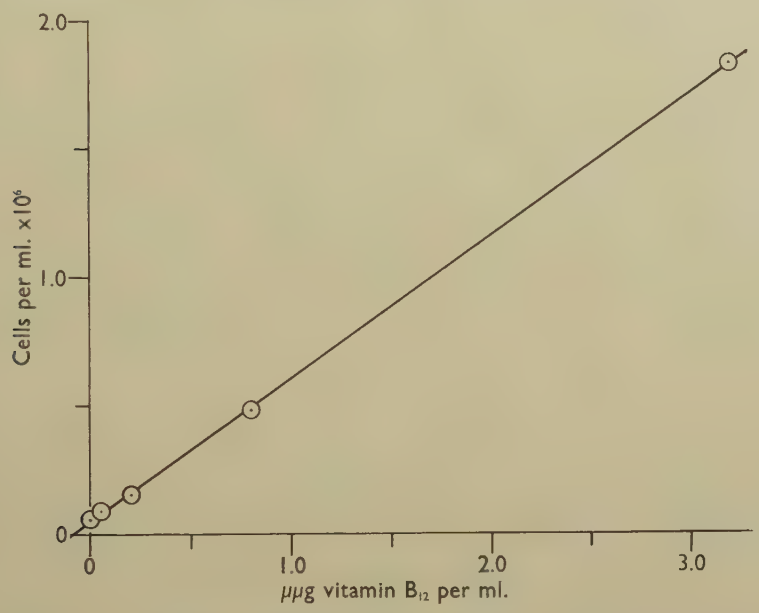

Fig. 3. 15° C experiment: dosage/yield plot on linear co-ordinates (27th day).

It is necessary to consider whether this result is a real one, whether in fact the concentrations measured (corrected dosage) were actually in force over the relevant period or whether the cells were sufficiently replete with the vitamin for any external concentration to be without influence, at any rate for a division or two. It will be recalled that the vitamin-depleted inoculum was small enough for there to be an estimated six or seven generations between inoculation and the earliest counts. Seven generations would effectively dissipate a hundred-fold surplus of intracellular vitamin in the unlikely event of there being one. Therefore it is improbable that the cells contained more than their necessary minimum of vitamin at the time of counting.

25° is near the limit of temperature tolerance of *Monochrysis* and several

cultures failed to grow at this temperature. Growth rate was significantly depressed in most of those that did grow, the depression increasing with decreasing vitamin concentration. However, the law apparently followed was not that expressed by Monod's equation, for no values for the constants c_1 and $k_{max.}$ could produce a curve to pass near all the points. It is probable that the concentration effect at the limiting temperature is real, but the data cannot be regarded as sufficiently precise to characterize it.

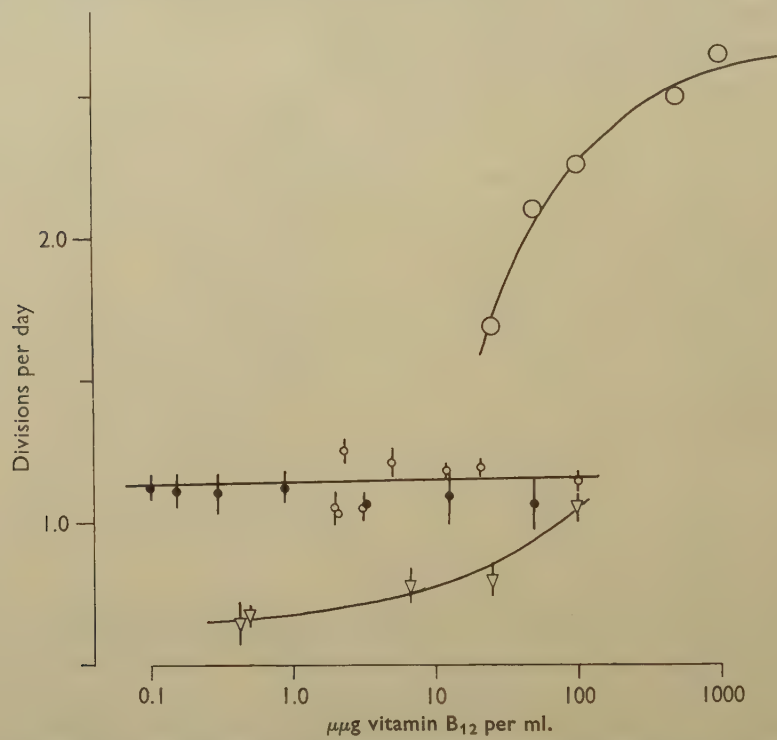

Fig. 4. Influence of vitamin B_{12} concentration (corrected dosage) on the initial rate of division in *Monochrysis*. (Three experiments: filled circles, 15° C; small open circles, 20° C; triangles, 25° C. 95 % fiducial limits indicated.) Large open circles: the same, in *Ochromonas* for comparison (data from Ford, 1958).

DISCUSSION

Comparison with Ochromonas

Ford's data on *Ochromonas* referred to earlier are also shown in Fig. 4. The contrast with *Monochrysis* is very marked: division rate is sensitive to vitamin B_{12} concentrations up to 100 $\mu\mu$g/ml. (c_1 = 13 $\mu\mu$g/ml.) and the maximum rate is over twice that of *Monochrysis*. There are some differences in technique which, however, are a consequence rather than a cause of the differences in

behaviour of the two organisms. *Ochromonas* is an obligate chemotroph, and Ford's experiments were carried out in the dark with a medium containing glucose as energy source. The normal upper temperature limit in *Ochromonas* is 33° C. Ford used one of 29° C.—well below the limit. Higher temperatures, if tolerated, may be expected to yield higher maximum division rates. Similarly, freedom from the limitations of phototrophic carbon assimilation allows higher over-all rates. *Monochrysis* is restricted as both to temperature and mode of carbon assimilation. It is possible that if these barriers could be overcome *Monochrysis* might behave more like *Ochromonas* in its requirement for vitamin B_{12}. The anomolous results in the 25° experiment invite further investigation along lines indicated by Hutner *et al.* (1957) and Scher & Aaronson (1958) for *Ochromonas* and *Euglena*. On the other hand, extrapolation of Ford's curve to lower vitamin B_{12} values brings it well below the level of the *Monochrysis* curve at the same values. Therefore, although it is clear that it was not limited by vitamin B_{12} concentration, *Monochrysis* has a higher potential for taking up the vitamin than *Ochromonas*.

A rough calculation shows that at the 'limiting concentration' of 13 $\mu\mu$g/ml. *Ochromonas* is picking up a molecule of vitamin B_{12} every 1·2–3·6 min from a medium containing about 200 molecules within a radius of 20 μ of the cell. By the same reckoning *Monochrysis* would appear to be able to take up the vitamin at a similar rate (a molecule every 2·4 min) from a medium containing as few as two molecules within the same radius.[1]

Ecological implications

If *Ochromonas* is typical of vitamin B_{12}-requiring marine phytoplankton then it may be safely concluded that vitamin B_{12} is indeed vital to phytoplankton economy. But if *Monochrysis* is typical then my original conclusions, namely that significant vitamin B_{12} concentrations are rather lower than the lowest yet measured in the sea, would still appear to be valid. That *Ochromonas* would live phagotrophically in nature and be independent of external concentration of nutrients is not relevant to the argument, but it is difficult to avoid drawing conclusions from the fact that the fast chemotrophic growth in Ford's experiments is not typical of phytoplankton activity. The problem, however, must remain open, at any rate until it becomes possible to study vitamin B_{12}-requiring members of the phytoplankton as precisely as these Chrysophyceae.

[1] Calculated from the following: *Ochromonas*, cell volume 250 μ^3, vitamin requirement 1–3 molecules per μ^3 of living alga, division rate 1·5 per day when vitamin concentration is 13 $\mu\mu$g/ml. (Ford, 1958). *Monochrysis*, cell volume 200 μ^3, vitamin requirement 3 molecules per μ^3, division rate 1·1 in vitamin concentrations as low as 0·1 $\mu\mu$g/ml. Molecular weight of vitamin B_{12} *c.* 1400.

SUMMARY

Yield of the supra-littoral chrysomonad *Monochrysis lutheri* varied directly as vitamin B_{12} concentration between the limits 0·1 and 100 $\mu\mu$g/ml., each $\mu\mu$g of vitamin supporting $0{\cdot}55 \times 10^6$ cells. Between the same limits of concentration there was to be seen no significant deviation in initial rate of cell division from the mean value of 1·1 divisions per day (phototrophic growth: 2000 lux, 20° and 15° C), the conclusion being that limitation of the division rate does not occur before the concentration vitamin B_{12} has dropped below 0·1 $\mu\mu$g/ml.

The behaviour of *Monochrysis* is compared with that of *Ochromonas*, and some ecological implications are discussed.

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A PRESSURIZING DEVICE FOR A CAMERA CASE DESIGNED FOR USE IN SHALLOW WATER

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(Plate I and Text-fig. 1)

With the exception of a few specially designed models, most under-water cameras today employ no means of pressurizing, or at best merely have a Schrader valve fitted to the case which can then be pressurized, prior to each dive, with a few strokes of a bicycle pump. This method has one severe drawback in that the majority of camera cases are better able to withstand external rather than internal pressure. The chances of a case exploding or blowing a gasket while pressurized prior to a dive are far greater than those of implosion of a non-pressurized case in a comparable depth of water.

Since pressurization is desirable, particularly in camera cases made of Perspex, the logical solution is a pressurizing device working on the same principle as the aqualung. This would permit the case to be pumped up automatically as it was taken into deeper water and thus never be subject to the chance of explosion or implosion. An aqualung, however, requires a self-contained source of air, and for a small camera case the cylinder must not be too large. It was found that Sparklet bulbs containing compressed CO_2 for making soda water were a convenient size and contained approximately 3 l. of gas. The case in question is designed for an Agfa Silette 35 mm camera and has a volume of about 1 l. One Sparklet bulb therefore permits pressurizing down to depths approaching 30 m.

The total pressurizing unit consists of a small demand-valve, a simple on/off valve (Fig. 1 *o.o.v.*) taken from a butane picnic stove, a brass adaptor (*a*) and a Sparklet bulb holder (*b.h.*) carrying the Sparklet bulb (*S.b.*). The adaptor has two functions: initially it enables the bulb holder to be screwed on to the rest of the unit as the threads on the holder and on the on/off valve are not identical; secondly it permits a gas tight union to be made between the Sparklet bulb and the on/off valve. The seal between bulb and adaptor is made by a small length of P.V.C. tubing fitted over the neck of the bulb which is compressed into the tapering orifice of the adaptor. The seal between the adaptor and the on/off valve is made with hemp and jointing compound. The pressure in the bulb is initially very high, being in the region of 4500 lb./sq.in., but the seals described have proved satisfactory. The Sparklet bulb is broached

by a steel needle (*n*) located at the inlet of the on/off valve, and the adaptor is of such dimensions that the steel needle just pierces the soft cap of the bulb when the holder is screwed fully home. The outlet of the on/off valve is threaded and screws into the inlet of the demand-valve, the seal being made with hemp and jointing compound and a lead washer.

The demand-valve is adapted from pieces taken from a Siebe–Gorman 'Salvus' breathing set. It is made of brass and consists of an irregular cylindrical chamber open at both ends. Through one side of the chamber comes the inlet tube (*i.j.*) which extends to the middle of the chamber and has an orifice in the form of a jet on its lower side. The larger, upper end of the chamber is sealed from the outside by a strong rubber diaphragm (*d.v.d.*) held in place by a threaded ring (*r*). Attached to the diaphragm is a brass cylinder (*c.c.*) threaded on the inside, and with a slot in it to fit over the inlet tube. The cylinder is free to slide up and down the chamber, its movement being limited by the flexibility of the diaphragm. A brass plug (*s.l.p.*) with a piece of P.V.C. glued to its face and a square lug on the back is screwed into this cylinder from the lower end until the P.V.C. is about 1 mm from the inlet jet. The plug is prevented from altering its setting by a washer (*w*) with a square hole and a single lateral projection which locates with the slot in the cylinder. A spring between this washer and the cap closing the lower end of the chamber forces the sliding cylinder up the chamber and presses the P.V.C. pad (*p*) against the inlet jet thus sealing it. An outlet (*o*) leads from a point in the chamber opposite the inlet and is connected to the camera case by a short length of polythene tubing. In addition there is a rubber spear valve (*s.v.*) fitted to this side of the system which acts as an exhaust valve.

As the case is taken into the water, increased pressure causes depression of the demand-valve diaphragm which pushes the sliding cylinder down the valve chamber and uncovers the inlet jet. Gas enters until the pressure inside the chamber (and by direct connexion, inside the camera case) is sufficient to raise the diaphragm and close the jet. This process continues as the case is taken deeper until the gas supply is exhausted. On surfacing the gas in the case expands and bubbles out through the spear valve exhaust. The depth at which the valve first opens can be altered by varying the compression on the spring at the base of the valve chamber. In practice it has been found convenient to have the valve set to open at a depth of about 2 m as this eliminates unnecessary wastage of gas due to minor fluctuations in depth during the course of a dive. Theoretically a gas reserve of 3 l. will permit up to three dives of 10 m or one dive of 30 m. The purpose of the on/off valve is to shut off the supply of gas between two shallow dives and thus reduce the strain on the demand valve.

Prior to fitting the pressurizing device it was found that at a depth of 10 or 12 m there was sufficient distortion of the camera case to permit the influx of water around the lid, which invariably resulted in failure of the 'flash'

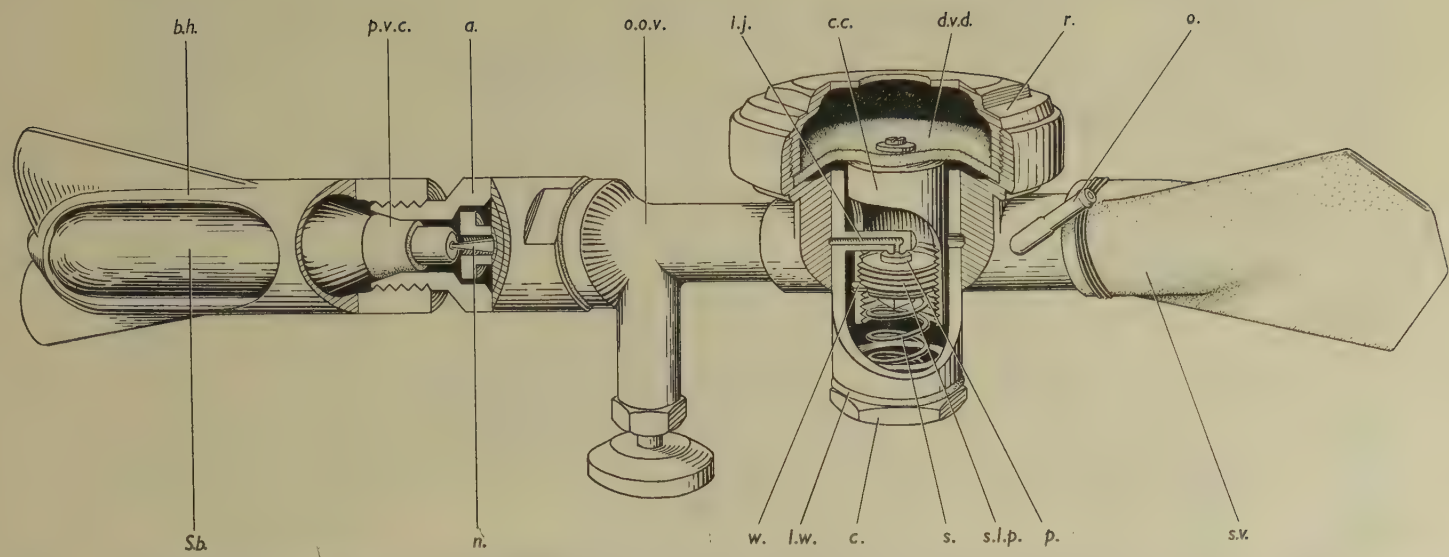

Fig. 1. Semi-diagrammatic cut-away view of the pressurizing device. *S.b.*, Sparklet bulb; *b.h.*, Sparklet bulb holder; *p.v.c.*, small piece of p.v.c.tubing acting as a gas-tight seal; *a*, adaptor; *n*, steel needle for piercing Sparklet bulb; *o.o.v.*, on/off valve; *i.j.*, demand-valve inlet jet; *c.c.*, central cylinder of the demand-valve; *d.v.d.*, demand-valve diaphragm; *p*, small p.v.c. pad; *s.l.p.*, square-lugged plug; *w*, washer with square hole and a lateral projection; *s*, spring; *l.w.*, leather washer effecting seal; *c*, cap closing lower end of the demand-valve; *o*, outlet to the camera case; *s.v.*, spear valve.

equipment. These setbacks have been experienced by other divers using non-pressurized cameras (G. R. Forster, personal communication). Since fitting the pressurizing device the camera has been used successfully to 26 m and there has been no flooding or failure of any part of the equipment.

The work was done at the Plymouth Laboratory, during the tenure of a D.S.I.R. Post-Graduate Studentship.

I am indebted to Mr F. J. Warren and the workshop staff for their advice during the construction of the apparatus, to Mrs E. A. Peace for the photograph, to Mr G. A. W. Battin for his assistance with the drawing, and to Mr G. R. Forster for his helpful criticism of the manuscript.

SUMMARY

A pressurizing device for an under-water camera is described. It consists of a single stage demand-valve working on the same principle as the aqualung, and a Sparklet bulb fitted to the demand-valve by an adaptor and an on/off valve is used as a gas supply. It permits automatic pressurizing of a camera case with a volume of 1 l. down to a depth of 30 m, and also permits the pressure to be released as the case is brought to the surface.

EXPLANATION OF PLATE I

Rear view of the camera case showing the pressurizing device in position, and the connexion between the case and the demand-valve.

(*Facing p.* 80)

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THE BEHAVIOUR OF *MACOMA BALTHICA* (L.)

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(Plate I and Text-figs. 1–3)

As is well known, not only sedentary animals such as barnacles and tubicolous polychaetes, but also motile animals like gastropod molluscs and various crustaceans, occupy characteristic and often rather narrow belts or zones on the shore which it must be assumed usually represent optimal conditions for the species. It is clearly of advantage if the behaviour of a motile species tends to confine the population to its zone, nevertheless leaving it free to explore its surroundings. Such movements may be so precise as to warrant the term 'homing' as in limpets (reviewed by Thorpe, 1956), less definite as in *Littorina littorea* (Newell, 1958*a*, *b*), or a combination of kineses and taxes as in *Lepidochitona cinerea* (Evans, 1951). Such behaviour results in the animal returning, after a period of mobility, to approximately its original position, thereby retaining its station on the shore. Most intertidal lamellibranchs are usually assumed to be completely sedentary or at best sluggish creatures rarely, if ever, making other than vertical movements, though exceptions are known. Orientated movements by lamellibranchs have received little attention, though Morton (1960) has studied those of *Lasaea rubra*, and asserts that this small creature is 'unusual among eulamellibranchs in its relatively great mobility on the surface of the substrate'. The present paper describes the movements of a small tellinid, *Macoma balthica*, most of the observations being made at Whitstable. As noted by various workers, for example Fraser (1932), Beanland (1940), Spooner & Moore (1940), Brady (1943), Holme (1949), and Smidt (1951), this species is often widely spread or even abundant on intertidal mud flats, usually distributed in a broad belt at and about mid-tide level. The animal lies just below the surface, maintaining contact with it by means of the separate inhalant and exhalant siphons.

In his classic review of the structure and adaptations of the Tellinacea, Yonge (1949) describes the feeding of *Macoma balthica*, classified by him as a non-selective deposit feeder. The animal extends its long inhalant siphon on the surface of the sand, actively drawing in all superficial deposits, and from time to time forcibly rejecting unsuitable material through it. The exhalant siphon is shorter and held vertically. Wernstedt (1942) has shown that *Macoma* can utilize both diatoms and detritus as food, and there can be little doubt that on mud-flats the bulk of the nutriment lies on the surface, in the form of naviculoid diatoms, deposited plankton, and detritus. When the

flats at Whitstable are uncovered by the tide the animals can be seen feeding on the surface deposits, attention being attracted by the very great activity of the inhalant siphon as it bends first in one direction, then in another, rapidly clearing the superficial layers from all around over an area as great as 4 cm. in diameter. When the tide is in, however, fresh supplies of food are continually brought to the animal, and it seems that then *Macoma* can behave as a suspension feeder, using the plankton as a source of food. Indeed in the laboratory *Macoma* frequently remain suspension feeding for several hours, presumably deriving sufficient food in this way. This supports the opinion of Hunt (1925) that generalized deposit feeders, with long free siphons, 'probably feed to some extent by drawing in water with suspended matter after the fashion of the short-siphoned bivalves'. During such activity the siphons are much shorter and practically immobile, in marked contrast with their writhing when deposit-feeding. Moreover, even at low water at Whitstable by no means all the *Macoma* are deposit-feeding, whereas at Cleethorpes where plankton is sparser the vast majority have been seen to be feeding on the superficial deposits. Perhaps what influences the method of feeding is the total amount of nutriment derived during a tidal cycle. There is evidence that an increase in light intensity, such as that experienced by the animals when the tide leaves them, induces a change from suspension to deposit feeding. This has repeatedly been seen in the laboratory, and it may be supposed that generally *Macoma* is a deposit feeder at low water and a suspension feeder when covered by the tide.

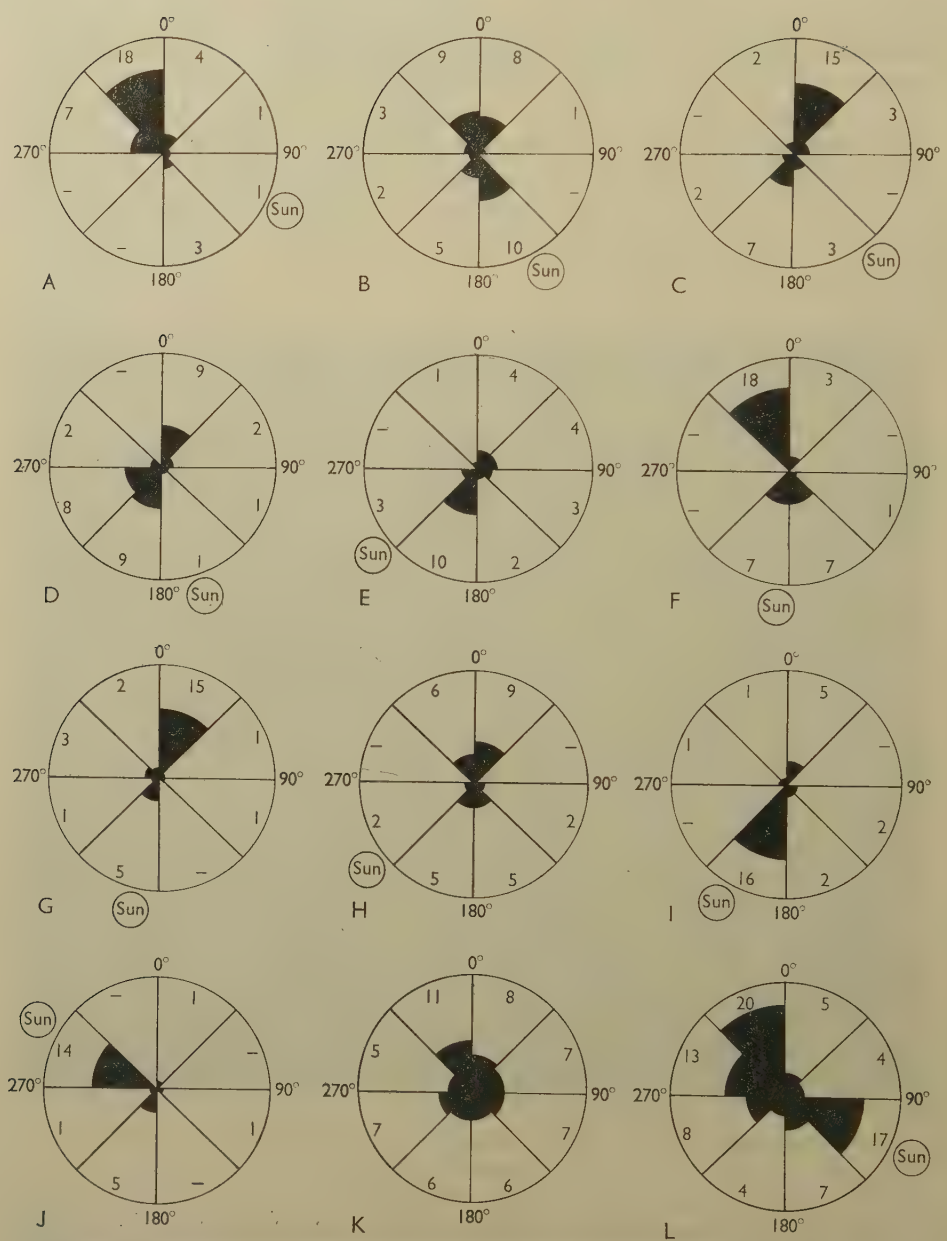
Macoma quickly clears the area around it of superficial material when deposit-feeding. If a deposit feeder as voracious as this is to continue to feed profitably until the tide returns it must move in order to tap fresh resources, and the same must apply, although to a lesser extent, to suspension feeders (e.g. *Cardium*) living in a restricted volume of water such as the small pools between ripple marks on mud flats. Obvious as the advantage of such movements would seem to be they have never been recorded. Yet they do take place, and tracks left by the animals, as furrows in the sand, are usually conspicuous on shores where *Macoma* is plentiful (Text-fig. 1), though animals are rarely seen in the act of moving. One which was observed, however, behaved in the following manner. At regular intervals of 15 sec the foot was extended forwards and then moved so as to thrust the animal, lying partially on its side, through the sand. A definite furrow about 5 mm wide was left on the surface. During movement the tips of the siphons could be seen at the base of the furrow. The foot contracted 15 times in 4 min, carrying the animal through a distance of about 5 cm. Another specimen, on a warmer day, moved at 5 sec intervals. On some sands, particularly in rough weather, tracks can be difficult to see.

The majority of the furrows seen are straight or nearly so, some are curved or U-shaped, and a few almost circular (Plate I). On several occasions on the

shore the directions of the straight tracks were measured by means of a prismatic compass, and the results grouped in octants and plotted as polar diagrams (Text-fig. 2). From these it is apparent that on sunny days there was a strong tendency for tracks to lie towards or away from the sun. On a completely overcast day, however, the directions were at random (Text-fig. 2 K). These results strongly suggest that the animals orientate to the direction of the sun's rays. Indeed there is no other environmental cue which could lead to orientation, for gravitational responses to a slope of less than 1 in 600 are hardly feasible.

Text-fig. 1. Tracks made by *Macoma balthica* in firm intertidal mud at Cleethorpes, Lincolnshire.

A hypothesis which would account for the form and orientation of the tracks is that any given animal, having been photopositive or photonegative for a time, later reverses the sign of response. In so doing it may exactly retrace its path, or describe an open or closed loop. Examples of all these types have been seen. It remains to account for the fact that the majority of



Text-fig. 2. Polar diagrams compiled by grouping the directions of straight furrows made by *Macoma* into octants. Figures indicate the numbers of tracks lying in each octant. The sun was clearly visible in all cases except K, when there was a heavy cloud cover and light was non-directional. The observations were made at various times of day, as indicated by the position of the sun. The *Macoma* zone had not been exposed for the same time in all instances.

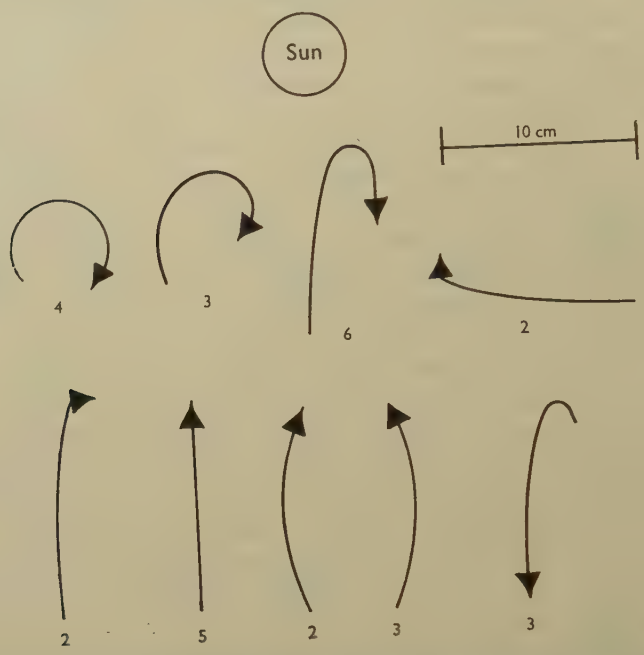
tracks seem straight, perhaps only slightly curved at their ends. This we believe to be due to one or more of three causes (a straight furrow representing one limb of a complete U-shaped path). First, the animals may have made their outward migration while still covered by sufficient water to obliterate the track, with the result that only the second limb remained visible. Certainly some tracks are completed very soon after the tide has withdrawn. On one occasion thirty tracks remained unchanged through an hour's observation some 2 h after the time of tide withdrawal. Secondly, the first-formed limb may have been obliterated by adverse weather conditions or looseness of the substratum. Thirdly, the second limb may not have been completed when observations were made, for *Macoma* has been seen moving at least 2 h after being uncovered by the tide. Probably all three have an effect in hindering the accumulation of clear cut results on the shore. It is not easy to assess the significance of these possibilities, since the animals often pause in their movements for long periods, making it difficult to follow a particular specimen through the whole course of its sequence of movement. In order to get further information on the typical form of a completed track, however, the beach was visited as the tide was receding, and tracks were carefully scrutinized as they appeared, when it was noticed that not only were the vast majority of the tracks U-shaped, but that the first-formed limb recorded a movement towards the sun. In many of the tracks, as might be expected, the photopositive limb was far less distinct than the other and could well have escaped notice previously, and indeed on this occasion unless particular attention had been focused on it. Observed tracks are schematized in Text-fig. 3.

Indirect support for the view that *Macoma* first has a photopositive phase of movement, followed by a photonegative one, comes from the fact that two strongly photopositive sets of data shown in Text-fig. 2 (I and J) were compiled when the *Macoma* zone had been exposed by the tide for an hour only; whereas a strongly photonegative set (A) was recorded after 5 h exposure.

Convincing as the field observations are that *Macoma* orientates by the sun, it would have been interesting to confirm them by laboratory experiments under controlled conditions. Unfortunately only rarely could specimens be induced to move under strong unidirectional light. Evans (1951) experienced the same difficulty with *Lepidochitona*. The few *Macoma* that were seen to move, however, did so in a manner similar to that on the beach.

From time to time tracks formed by *Cardium edule* have been seen. On one occasion, of fourteen such tracks observed, thirteen were U-shaped, with the first formed limb towards the sun and the second away from it. Two tracks formed by *Venus striatula* have been seen at Kames Bay, Millport. Both were straight or nearly so, and directed away from the sun. *Littorina littorea* is known to make light orientated movements (Newell, 1958*a*, *b*), as is *Hydrobia ulvae* in one phase of its activity (Newell, 1961). Linke (1939) gives photographs of looped tracks made by *Bathyporeia robertsoni*, *Pygospio*

elegans, and *Corophium volutator*. Further observations on suitable animals may furnish more examples of the phenomenon. Such movements, whose ecological significance seems to be that the animal can tap fresh food resources while maintaining its station on the shore, may prove to be far more widespread among intertidal animals than previously thought.



Text-fig. 3. *Macoma* tracks schematized from thirty observations made at Whitstable immediately after tide withdrawal. Figures indicate the numbers of each type seen.

SUMMARY

The tellinid *Macoma balthica* moves extensively about the surface of the sand, its activities not being restricted to vertical movements as hitherto supposed.

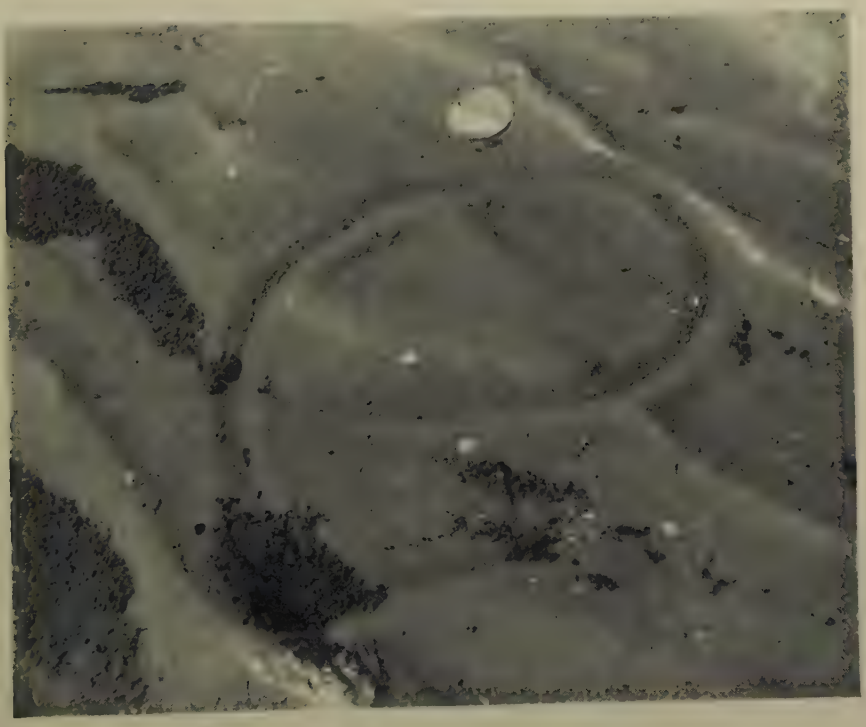
These horizontal migrations comprise a movement towards the sun followed by a movement away from it. If there is heavy cloud movements still occur, in summer at least, but are at random.

Other intertidal species indulge in similar movements, possibly indicating a widespread habit which confers the advantage of bringing the animal into contact with surrounding areas while maintaining its station on the shore.

EXPLANATION OF PLATE I

A. A typical U-shaped track of *Macoma*, from the sand at Whitstable, Kent.
B. A closed looped track of *Macoma*, from Whitstable, Kent.

A



B

(*Facing p.* 86)

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THE GENERIC POSITION OF THE BRACHIOPOD *MEGERLIA ECHINATA* (FISCHER & OEHLERT)

By D. Atkins, D.Sc.
From the Plymouth Laboratory

(Text-figs. 1–4)

The genus *Pantellaria* was created by Dall in 1919 for *Megerlia* (= *Terebratula*) *monstrosa* (Scacchi) and *M.* (= *Mühlfeldtia*) *echinata* (Fischer & Oehlert), because he considered that 'the opening for the pedicle in intact specimens is wholly in the flat or sessile valve, as in *Platidia*; when a portion of it appears to involve the other valve it is due to wear'. Dall seems to have been confused over *Platidia*, for Thomson (1927) described the foramen in that genus as amphithyrid, as indeed it is. Dall went into the matter rather more fully in 1921 and his reasons for creating the new genus were summarized by Thomson (1921) as follows: '*Pantellaria* differs from *Mühlfeldtia* only in possessing an amphithyrid instead of a submesothyrid foramen, and in the flattening of the dorsal valve, together with its lack of ornament.' Thomson remarked that 'in view of the marked polymorphism of *Mühlfeldtia truncata* according to its mode of support, it is possible that the attainment of amphithyrid beak characters may in this case be rather of specific than of generic value, unless as in *Amphithyris*, it is found to be a character of very early appearance in development'.

Megerlia monstrosa (Scacchi), which Dall made the genotype of his new genus *Pantellaria*, has not been obtained at Plymouth, but a number of *M. echinata* (Fischer & Oehlert) of shell length 1·2 and width 1·3 mm to shell length 13·5 and width 18·5 mm were dredged by R.V. 'Sarsia' in May 1958 and July 1959. These have allowed of a study of the young stages of the shell and the mode of development of the pedicle opening of the adult.

The dorsal valve of *M. echinata* is closely applied to the substratum and externally the adult shell somewhat resembles a large *Platidia davidsoni* (Eudes Deslongchamps). If first the change in shell shape with age is considered, it is seen that in the young of shell length rather more than 1 mm (Fig. 1) the dorsal valve is somewhat more convex than the ventral, especially posteriorly, although the ventral already bears spines and the dorsal is without them. The dorsal valve being closely applied to the substratum reproduces its irregularities, thus in some adult *M. echinata* this valve is flat and even slightly concave anteriorly, although tending to remain somewhat convex posteriorly, while the ventral valve is markedly convex (Figs. 3, 4). Occasion-

ally the dorsal valve is irregularly and rather deeply convex, fitting into a depression in the substratum. Some shells occupied the empty cups of coral polyps, had deeply convex dorsal valves, only slightly convex ventral valves and when large projected beyond the edge of the cup. In fact the degree of concavity of both valves of *M. echinata* varies greatly.

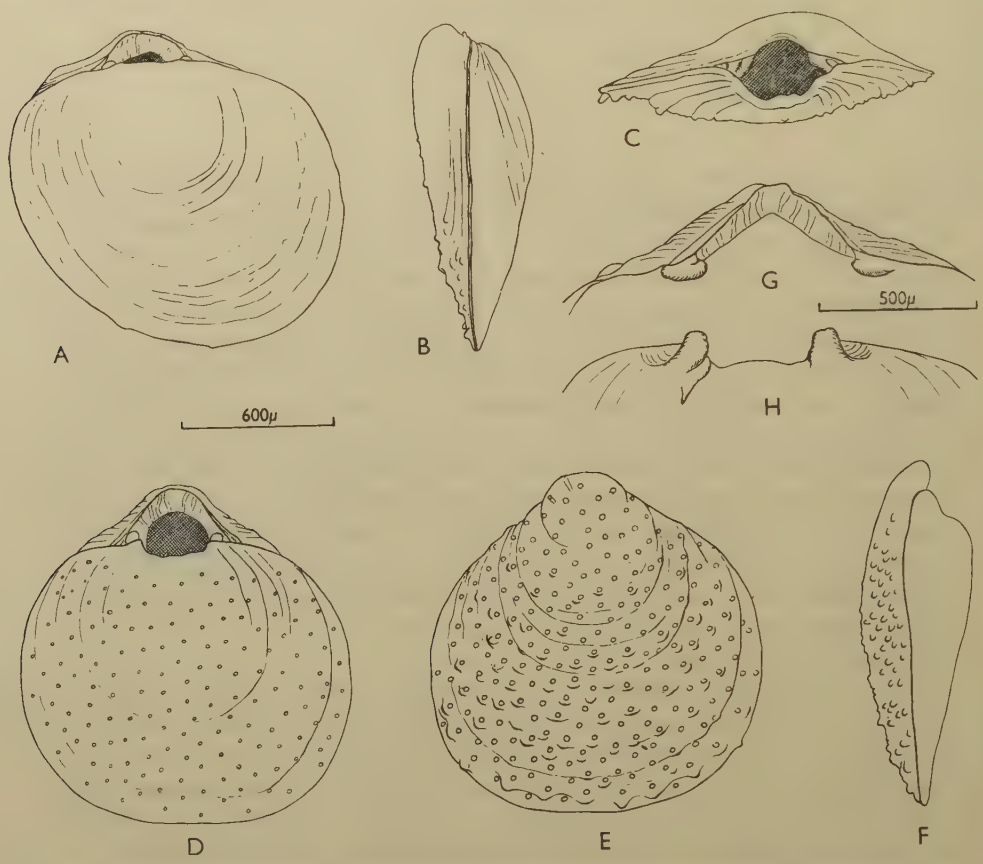

Fig. 1. *Megerlia echinata*. A, dorsal; B, lateral and C, posterior views of a specimen of shell length 1·2 mm and width 1·3 mm. D, dorsal; E, ventral and F, lateral views of a specimen of shell length 1·35 mm and width 1·32 mm. Mantle setae omitted. G, dorsal view of beak; and H, ventral view of cardinalia of a specimen of shell length 1·4 mm and width 1·6 mm; crus present on one side only.

The ventral valve has a radial ornamentation of spines which in some *M. echinata* may be worn down and only visible near the margins and occasionally may be absent. The irregular surface of the dorsal valve, besides showing growth lines may, as mentioned by Fischer & Oehlert (1891), have very indistinct radiating folds, often only visible anteriorly.

When on a smooth surface *M. echinata* is regularly transversely oval, but is sometimes most irregular in shape when attached to coral owing to the irregularities of the surface and in fact is more irregular than the figures of *M. monstrosa* by Fischer & Oehlert (1891, pl. vii, fig. 12). These irregularly shaped *M. echinata* are clearly spinous. Mantle setae are present, but have been omitted from the figures, except for Fig. 2. They reached a length of 1·8 mm in a specimen 1·6 mm long and 1·5 mm wide, and a length of 2 mm in an adult 9 mm long and 12 mm wide, but often setae are broken.

The prominence of the beak evidently varies in the young of about 1 mm shell length; in one 1·2 mm long it projected slightly (Fig. 1 A), whilst in another 1·35 mm long it was prominent (Fig. 1 D). The pedicle collar is short, and is free of the valve anteriorly: it appears striated in the young owing to the longitudinal direction of the pits. In adult specimens of shell length 9 mm and more the mid-region of the pedicle collar protrudes (Fig. 4 B, C), and does not follow the curve of the ventral valve. This protrusion of the collar is not found in *M. truncata*.

As previously mentioned, according to Dall (1921) in *Pantellaria* the pedicle opening is normally confined to the brachial (dorsal) valve, only by wear encroaching on the other. He considered that this encroachment on the ventral valve—as shown in most figures—was abnormal. That this is not so for *M. echinata* can be seen from immature shells 1·2–3·5 mm long (Figs. 1, 2).

In the smallest *M. echinata* seen, shell length 1·2 mm (Fig. 1 A) the posterior edge of the dorsal valve appeared to be without a notch in dorsal view. When viewed posteriorly (Fig. 1 C) it was evident that the valve was strongly convex posteriorly, and the part of the pedicle opening in it was a slight semi-circular notch, in a plane at right angles to that of the valve. (A notch in this plane has been seen in *M. truncata* of length 6 mm and width 7·2 mm.) It is only in adults in which the dorsal valve has become flattened that the part of the pedicle opening in it is a semi-circular notch in the plane of the valve (Fig. 4), as in *Platidia*. In *Platidia* the notch in the posterior edge of the dorsal valve is as relatively large, and in the same plane as the valve, in an animal of shell length 0·8 mm as in adults of 7 or 8 mm, and the valve is as flat in one as in the other (Atkins, 1959).

In *Megerlia truncata* the dorsal valve is less convex than the ventral and both are free and similarly sculptured. The foramen is described as submesothyrid (Thomson, 1927). The few living adult *M. truncata* seen were held by the very short pedicle at an angle to the substratum, the yellow coral, *Dendrophyllia cornigera* Lamarck, often almost vertically, particularly in large specimens. That the almost erect position is the usual one is supported by additional empty shells with both umbos badly worn. In *M. echinata* on the other hand the pedicle is almost at right angles to the shell, at least in large specimens. Internally *M. echinata* is very like *M. truncata*.

In those *M. echinata* in which the notch in the dorsal valve is small, the

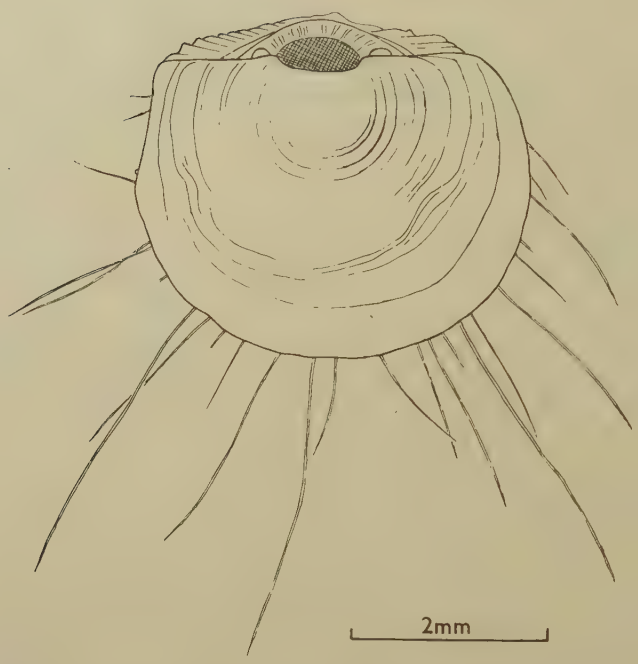

Fig. 2. *Megerlia echinata* of shell length 3·5 mm and width 3·9 mm; dorsal view, with such mantle setae as remain.

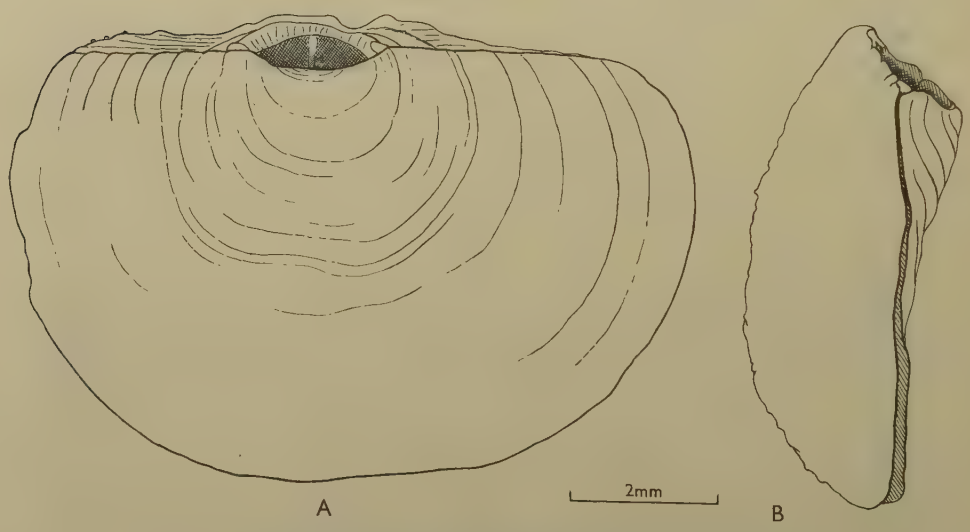

Fig. 3. *Megerlia echinata* of shell length 6·2 mm and width 8·7 mm. A, dorsal and B, lateral views.

crural bases are as in *M. truncata*, and have been figured by Fisher & Oehlert (Fischer & Oehlert, 1891, pl. vii, fig. 13*e*). But in those with wide semicircular notch the crural bases arising from the inner socket ridges are produced, following the curve of the semicircle (Atkins, 1961, fig. 16). They are excavate underneath; their ventral surfaces are hollowed posteriorly and serve for the attachment of the dorsal pedicle muscles.

I think it is evident from the foregoing description of immature shells that the form of the pedicle opening is of specific and not of generic value and that *Pantellaria echinata* should be transferred back to *Megerlia*.

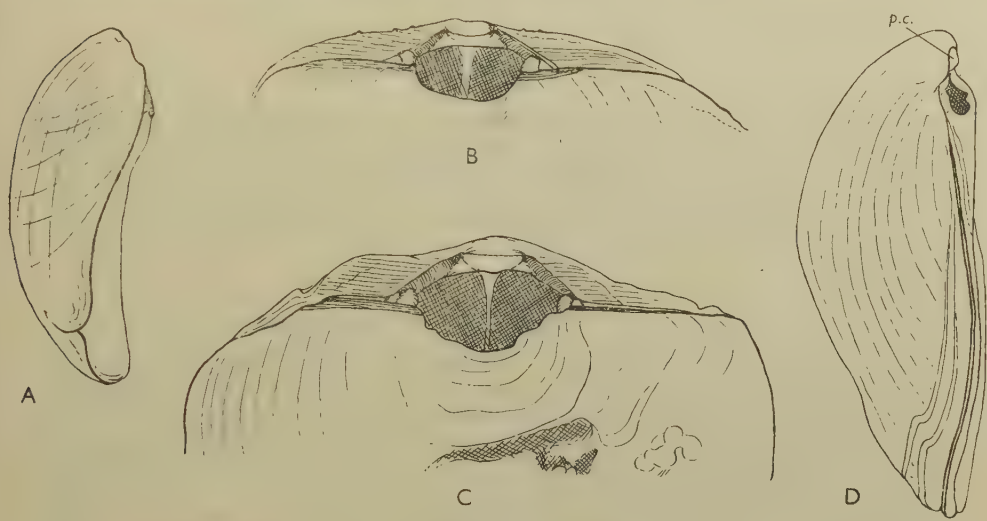

Fig. 4. *Megerlia echinata*, A and B of shell length 9·2 mm and width 13 mm; C and D of length 12 mm and width 15·6 mm. A and D lateral views; B and C posterior regions viewed dorsally. The spines of the ventral valves of these two specimens were small or abraded. Both show narrow cardinal areas on the dorsal valve, although partly abraded on the right side; the worn region is indicated by a broken line in B. In the shell shown in C, deltidial plates could not be distinguished. *p.c.*, pedicle collar.

M. echinata has much the appearance when viewed ventrally of the illustration given by Deshayes (1863) of his *Morrisia gigantea* from off the Isle of Reunion in 200 fathoms (wrongly given as 33 fathoms by Thomson, 1927). His single specimen was attached to a crustacean. He did not mention its mode of attachment, although as he identified it as a *Platidia* (= *Morrisia*) and mentioned that the pedicle opening was equally divided between the two valves, it is probable that it had the habit of that genus, the dorsal valve applied to the substratum. Deshayes said that 'les valves sont inégales et ornées a l'extérieur, surtout la plus grande, de costules étroites, peu épaisses et granuleuses; elles sont peu visibles sur la valve supérieure [i.e. dorsal]; examinée à la loupe, la surface montre une multitude de fine granulations.'

Dall (1921) tentatively placed *Morrisia gigantea* in *Megerlia* (= *Pantellaria*) *echinata*: it had previously been identified with *Megerlia truncata by* Jeffreys (1878) and Davidson (1887). Jackson (1921) was not convinced of Dall's identification, and was more inclined to refer *Morrisia gigantea* to his *Megerlia truncata* var. *paucistriata* from the Persian Gulf. After examining Jackson's *Megerlia* from this locality I would be inclined to agree rather with Dall's view than that of Jackson, but identification can only be tentative without seeing Deshayes' shell.

I am indebted to Mr G. F. Elliott for discussion.

SUMMARY

The variation in convexity of the valves and the development of the pedicle opening in *M. echinata* is described in shells from a length of 1·2 mm to 13·5 mm and it is concluded that the differences in shell shape and form of the pedicle opening between *M. truncata* and *echinata* are of no more than specific value and that the latter species should be transferred from *Pantellaria* back to *Megerlia*.

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THE GROWTH STAGES AND ADULT STRUCTURE OF THE LOPHOPHORE OF THE BRACHIOPODS *MEGERLIA TRUNCATA* (L.) AND *M. ECHINATA* (FISCHER & OEHLERT)

By D. Atkins, D.Sc.
From the Plymouth Laboratory

(Text-figs. 1–16)

Two species of *Megerlia*, *truncata* and *echinata*, have been dredged by R.V. 'Sarsia' in recent years, allowing of a study of the growth stages of their lophophores and of the feeding mechanism. The generic position of *M. echinata* has already been discussed (Atkins, 1961 *a*).

MEGERLIA TRUNCATA (L.)

Megerlia truncata was dredged in February and July 1956, and again in June 1957, from a small area of yellow coral, *Dendrophyllia cornigera* Lamarck, off the Point of Penmarch at a depth of 45–48 fathoms. They were attached to the dead region of the growing coral, in company with *Terebratulina retusa* (L.) and *Platidia davidsoni* (Eudes Deslongchamps). They varied in size from a shell length of 1·5 mm and width of 1·2 mm to a shell length of 16 mm and width of 18 mm. Fischer & Oehlert (1891) gave the length as 9 mm and width 11 mm, whereas Davidson (1887) gave the length as 9 lines and width 10 lines, which is about 19 mm by 21 mm. The shell of the young is longer than wide, but this relationship changes with increased size.

The pedicle opening is large and submesothyrid (Thomson, 1927). However, in two individuals of length 2·8 and 4·3 mm, a shallow semicircular notch was present in the dorsal valve (Figs. 3, 5), and in one of length 6 mm and width 7·2 mm the semicircular notch was in a plane at right angles to that of the valve. The shell of length 2·8 mm and width 3·1 mm was not flattened on to the substratum, but stood almost vertically, as is usual in *Megerlia truncata*. The position of attachment of the other two was not especially noted.

The shell of adults is of a fulvous colour, that of the young whitish or yellowish. In the adult the flesh was of a dark orange colour: in a small specimen of length 3·9 mm, while the flesh was an orange tint, the digestive diverticula and gut were dark brown. Mantle setae were present in both young and adults; in the latter the follicles were orange tinted. Mantle setae appear especially long in the young: they have been omitted from all figures.

Between the mouth and the transverse band of the loop are two carmine pigment spots, such as noted in certain other brachiopods (Atkins, 1959*a*, *c*, 1961*b*). It may be recorded here that the posterior adductor muscles are striated.

Results of narcotizing *M. truncata* were variable, but on the whole young stages were difficult to relax successfully.

All figures in this and the following section on *M. echinata* were drawn with the aid of a camera lucida.

Growth stages of the lophophore and loop

Young stages of the brachidium of *M. truncata* at 2 and 4 mm shell length have been described and figured by Eudes Deslongchamps (1884) and by Fischer & Oehlert (1891) at 4 mm long, but the relationship of lophophore to loop has so far not been described in detail.

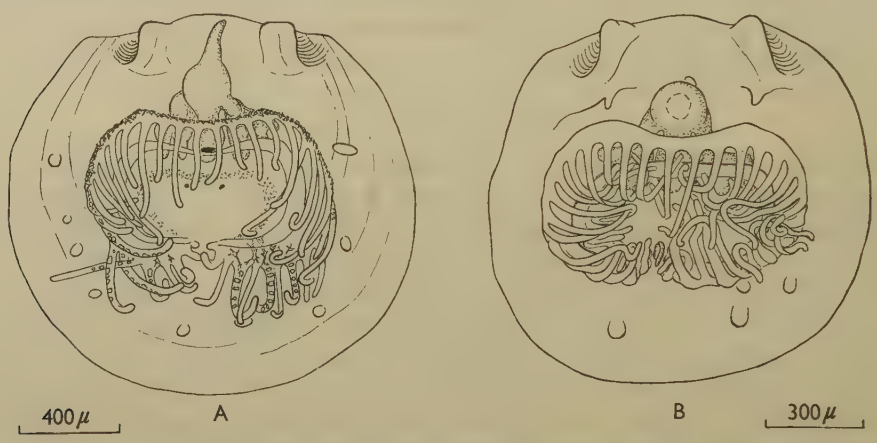

Fig. 1. *Megerlia truncata*. A. Brachial valve of a specimen of shell length and width 1·6 mm, drawn living, the very early schizolophe not fully expanded. The filaments behind the mouth are standing almost upright and therefore appear short. Spicules are indicated and a semicircle of mucous glands to the inner side of the food groove. The two carmine pigment spots are shown. Muscles and mantle setae omitted. B. Brachial valve of a specimen of shell length 1·5 mm and width 1·2 mm, drawn preserved after unsuccessful narcotizing. Although of smaller size than the specimen shown in A, the lophophore is somewhat more advanced. Small crura are present and a minute double septum at the anterior invagination of the lophophore. Spicules, mantle setae, muscles and carmine pigment spots omitted: intestine removed.

A number of immature *M. truncata* were found among material dredged by R.V. 'Sarsia' from off Penmarch, but they did not furnish a complete series of growth stages of the lophophore. A fairly closely gradated series of shell length 1·6–4·3 mm was obtained, but the earliest stages of the growth of the ring are unknown in this species as they apparently occur in individuals about 2·4–2·8 mm long and these were wanting. A large hiatus exists between

shell lengths 4·3 and 7 mm. At the latter size the lophophore is early plectolophous.

The earliest stage of the lophophore seen was very early schizolophous in a specimen of shell length and width 1·6 mm. Most of the filaments are in a single series with ridged frontal surfaces, although a few grooved filaments are present anteriorly (Fig. 1 A). The filaments are rather unequal in length; this is no doubt abnormal. Spicules support the base of the lophophore and are present in the outer filaments and in a few of the more anterior inner filaments. Crura and septum are not yet developed.

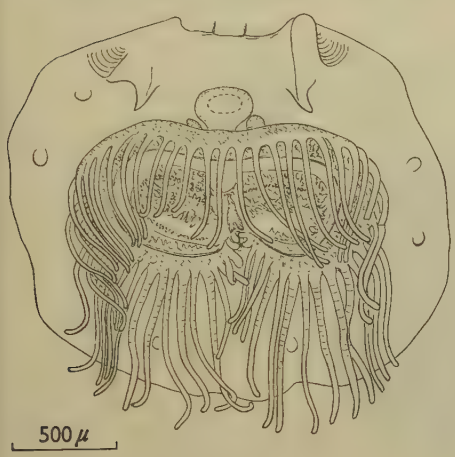

Fig. 2

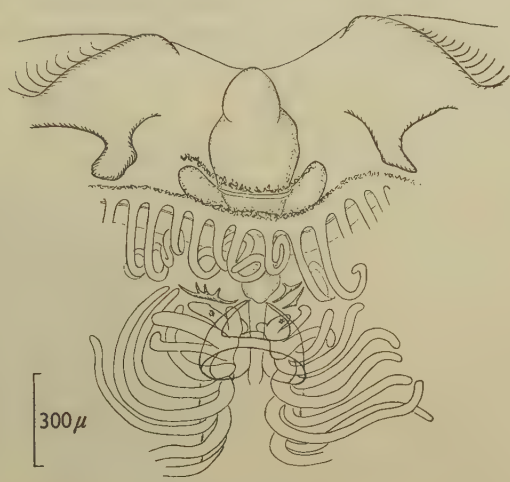

Fig. 3

Fig. 2. *Megerlia truncata* of shell length 2·4 mm and width 2·1 mm. Brachial valve with early schizolophe, showing some irregularity in the arrangement of the filaments. Spiculation is indicated and the band of mucous glands on the brachial membrane. The carmine pigment spots are hidden by overlying filaments. Intestine removed. Drawn living, narcotized.

Fig. 3. *Megerlia truncata* of shell length 2·8 mm and width 3·1 mm. Posterior region of the brachial valve to show the relation of the brachial support and the late schizolophe (somewhat contracted). The lophophore overlies most of the loop. Drawn living, the brachial support added after staining and clearing. Spicules mostly omitted.

In a slightly smaller *M. truncata* of shell length 1·6 mm and width 1·2 mm (Fig. 1 B), but with rather more advanced lophophore, minute processes arising from near the bases of the inner socket ridges represent the crura. What appears to be a minute double septum is present in the anterior invagination of the lophophore.

At a shell length of 2·4 mm and width of 2·1 mm there is an increase in the number of filaments and in the spiculation, although the lophophore is still early schizolophous (Fig. 2). This specimen shows anteriorly some irregularity in the arrangement of the two series of filaments, for on the left two

and on the right three outer filaments occur without intervening inner filaments. An elongated hood is borne on a slight median ridge.

The late schizolophous stage has been found at the following shell sizes: (1) length 2·8 mm and width 3·1 mm (Fig. 3); (2) length 3·4 mm and width 3·8 mm; and (3) length 3·5 mm and width 3·2 mm. The appearance of the brachial support, so far as can be discerned by clearing the brachial valve in cedar-wood oil, is of a ring, narrow ventrally and broadly attached dorsally to a low septum; it supports the blind end of the invagination of the lophophore (Fig. 3). The state of the ring at this shell size agrees with the stage depicted by Eudes Deslongchamps (1884, pl. vii, fig. 2) at a shell length of 2 mm, except that the anterior spinous processes shown by him are absent. His figure of

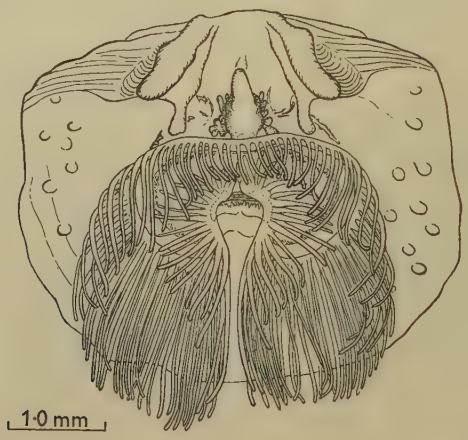

Fig. 4. *Megerlia truncata* of shell length 3·9 mm and width 4·5 mm. Brachial valve with early zygolophe, drawn living. Position of some spicules indicated. Muscles and mantle setae omitted.

the brachial support resembles that of *Terebratula lunifera* (Philippi, 1836, pl. vi, fig. 16*a*) at a shell length of $\frac{5}{4}'''$ and width of $1'''$. The cardinalia of *T. lunifera*, however, differ from those of *Megerlia truncata*, though this may have been due to inaccurate drawing. It was assumed, probably rightly, by Jeffreys (1878) that Philippi's *Terebratula lunifera* was the young of *Megerlia truncata*, the adult of which Philippi also figured.

The schizolophous lophophore shown in Fig. 3 is somewhat contracted and the filaments lining the median invagination are turned outwards. When well expanded the tips of these filaments approximate, roofing in an excurrent channel; this position of the filaments occurs only previous to deflexion of the lateral arms.

The early zygolophous stage of the lophophore, with lateral arms just beginning to be deflected, occurred in three individuals. In those of shell length 3·7 mm and width 3·9 mm, and shell length 3·9 mm and width

4·5 mm (Fig. 4) the crura are rather more developed than in the previous stage; the ring has increased in diameter, and shows laterally slight forward projections. In a somewhat larger specimen of shell length 4·3 mm and width 5·6 mm (Fig. 5) crural processes are obvious; the descending branches are short. The anterior extensions of the ring are more pronounced and have come to point outwards. As far as this stage the ring gives support to little more than the growth regions of the lophophore where spicules are few and development of supporting substance slight. The remainder of the lophophore is supported by heavy spiculation, not shown in Fig. 5. In *M. truncata* the descending branches of the loop are late in developing.

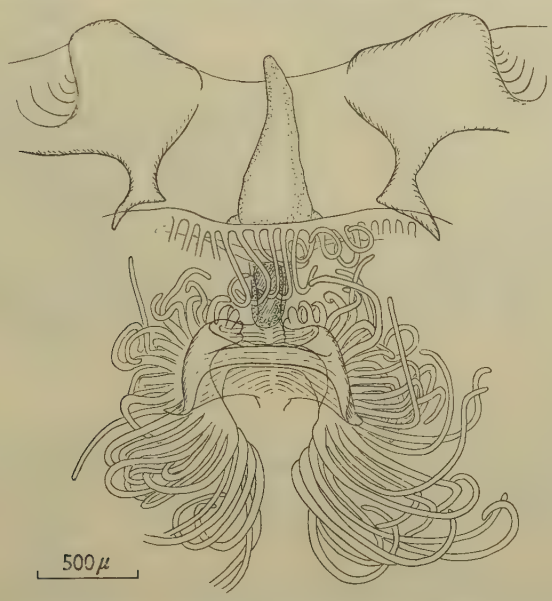

Fig. 5. *Megerlia truncata* of shell length 4·3 mm and width 5·6 mm. Posterior region of the brachial valve to show the relation of the brachial support and the zygolophe, with lateral arms just beginning to be deflected. The lophophore overlies most of the loop. The septum is continued posteriorly under the gut, but is obscured by the thickness of the stomach. Stained and cleared. Spicules omitted.

No *M. truncata* were obtained between a length of 4·3 and 7 mm. At a shell length of 7 mm and width of 9 mm the lophophore is early plectolophous with little more than one turn to the spiral arm. The descending branches are complete and the loop much as in the adult (Fig. 6A). At a shell length of 9 mm the spiral is somewhat more coiled (this individual is a male with small ripe gonad).

The adult lophophore

The adult lophophore is plectolophous. Owing to the generally short and broadly transverse shape of the shell the lateral arms are short and the spiral

arm short and broad (Fig. 6B). The fully adult loop has been figured by several authors and may be seen in Fischer & Oehlert (1891, pl. vii, fig. 11*g–i*). The relation of the lophophore to the body, and of the lateral and spiral arms to one another and to the brachial support in *M. truncata* of shell length 12 mm and width 15 mm is shown in transverse sections illustrated in Fig. 7. The organic connexion between the lateral and spiral arms is extensive, as may be seen from these sections; the lateral arm and the corresponding half of the spiral arm still have a common great brachial canal when the section is anterior to the brachial membrane connecting the two sides of the spiral

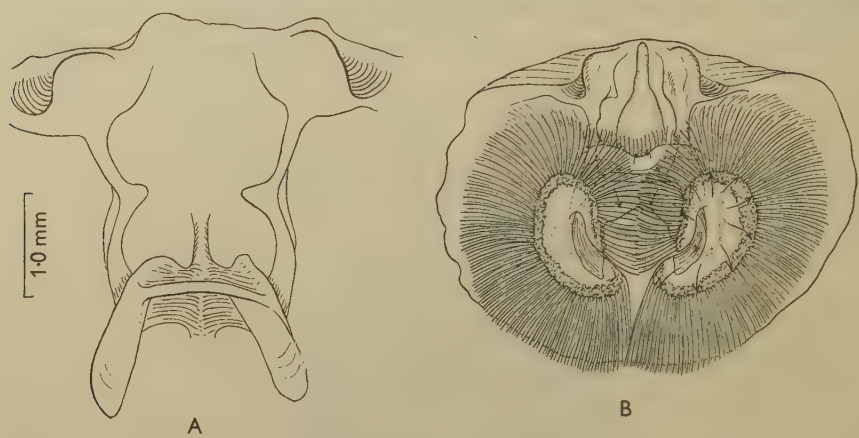

Fig. 6. *Megerlia truncata.* A. Brachial support of a specimen of shell length 7 mm and width 9 mm: the lophophore was in the plectolophous stage. Beak worn. B. Brachial valve with plectolophe of a specimen of shell length 15 mm and width 16 mm; drawn living. Spicules on the oral disc omitted, as also the mantle setae. Currents on the body of the lophophore shown by arrows. The scale applies to A only.

arm (Fig. 7D). The section shown in Fig. 7A is through the transverse band and the bands connecting it with the low median septum. Below the transverse band is an extension of the mantle cavity. The coelome extends into the lateral arms, and on the left is dividing off as the brachial pouch. A more anterior section (Fig. 7B) passes anterior to the transverse band and through the descending and ascending branches of the loop. Fig. 7C is of a section through the twin growth regions of the lophophore at the apex of the spiral arm. The brachial membrane connecting the two sides of the spiral arm is deeply depressed medianly, so that transverse sections through the anterior region of the arm show the two sides unconnected (Fig. 7D). This incurvature of the membrane is present in life (Fig. 6B).

The broad calcareous supporting loop does not reach to the extremities of the lateral arms (Fig. 6B). A broad band of spicules is present at the bases of the filaments. Between this basal band and the edge of the loop the membrane is transparent, with only a few spicules. Indeed it is so transparent that when

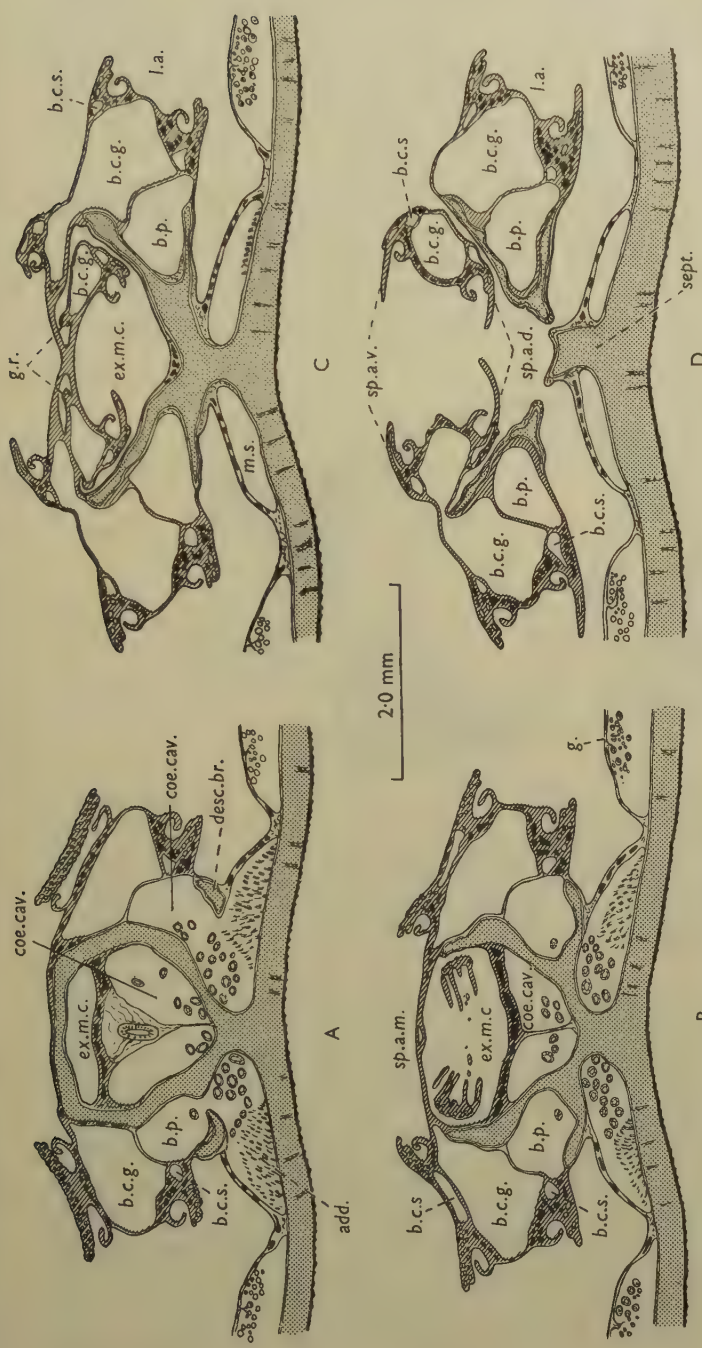

Fig. 7. *Megerlia truncata* of shell length 12 mm and width 15 mm. Transverse sections to show the relation of the lophophore to the body. A. Through the transverse band and the bands connecting it with the low median septum. On the left the brachial pouch is becoming separated from the coelomic cavity. B. Anterior to the transverse band. The posterior end of the spiral arm is present beneath the membrane connecting the two sides of the first coil of that arm. C. Through the growth regions of the lophophore, at the apex of the spiral arm. D. Through the anterior region of the spiral arm, the two sides of which are free from one another, the section being anterior to the connecting membrane. The lateral arms are free from the septum. *add.*, adductor muscle; *b.c.g.*, great and *b.c.s.*, small brachial canals; *b.p.*, brachial pouch; *coe.cav.*, coelomic cavity containing digestive diverticula; *desc.br.*, descending branch of the loop; *ex.m.c.*, extension of mantle cavity; *g.*, gonad; *g.r.*, growth regions; *l.a.*, lateral arm; *m.s.*, mantle sinus; *sept.*, septum; *sp.a.d.*, dorsal and *sp.a.v.*, ventral side of spiral arm; *sp.a.m.*, membrane connecting opposite sides of spiral arm. Spaces formerly occupied by spicules shown black; supporting substance hatched and shell and brachial support stippled.

the lophophore is viewed ventrally the basal band of spicules of the dorsal side of the arm is visible through the membrane. The bases of the ventral filaments of the lateral arms lie nearer the lateral edges of the shell than do those of the dorsal row. The membrane forming the floor of the spiral arm is transparent and contains only a few tiny spicules. The floor of the food groove and the base of the lip are heavily spiculated.

Small brachial canals, giving off branches to the filaments, and great brachial canals are present. A blood vessel runs in the small canals (Fig. 8). In each lateral arm a brachial pouch extends a short distance beyond the calcareous loop, but not to the extreme end of the arm. There appears to be some connexion between the length of the loop and degree of extension of the

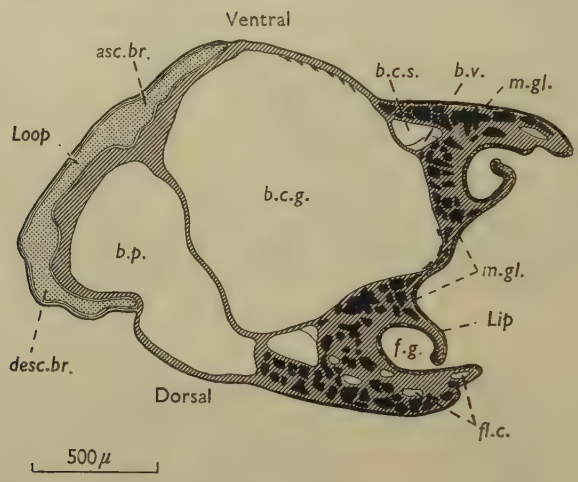

Fig. 8. *Megerlia truncata.* Transverse section through a lateral arm of a specimen of shell length 12 mm and width 15 mm. *b.v.*, blood vessel; *f.g.*, food groove; *fl.c.*, filamentar canal; *Lip*, lip of the food groove; *loop*, fused descending (*desc.br.*) and ascending (*asc.br.*) branches of the loop; *m.gl.*, mucous gland cells. Other lettering and shading as in Fig. 7.

coelome, as the brachial pouch, into the lateral arms, although as the loop is formed by an invagination of the outer epithelium (Williams, 1956) it is difficult to see the significance of the connexion. In *Macandrevia cranium* (Müller), *Terebratalia transversa* (G. B. Sowerby) and *Terebratella inconspicua* (G. B. Sowerby) in which the loop extends to the extreme tip of the arm, so does the brachial pouch (Atkins, 1959*b*, *c*, 1961*b*). In *Platidia davidsoni* (Eudes Deslongchamps) in which the descending branches of the loop are absent, the brachial pouches are extremely short; they can hardly be said to exist (Atkins, 1959*a*). In the short-looped *Terebratulina retusa* (L.) and *Gryphus vitreus* (Born) the brachial pouches extend but a short distance into the lateral arms (Atkins, unpublished).

Muscle fibres are present in the walls of the canals, chiefly beneath the

supporting substance under the food grooves where they are mainly transverse in direction. They are too fine to be shown well at the magnification of Fig. 8.

The filaments are in a double alternating series except for some 11 to 12 pairs behind the mouth in single series and with ridged frontal surfaces. The outer filaments are rather deeply grooved, and in an adult 15 mm long and 16 mm wide were about 0·85 mm wide near the base, while the inner filaments were about 0·5 mm wide. Longitudinal muscle fibres are present in the walls of the filamentar canals, being most strongly developed frontally and to a lesser degree abfrontally. Striated fibres appear to be restricted to the frontal group.

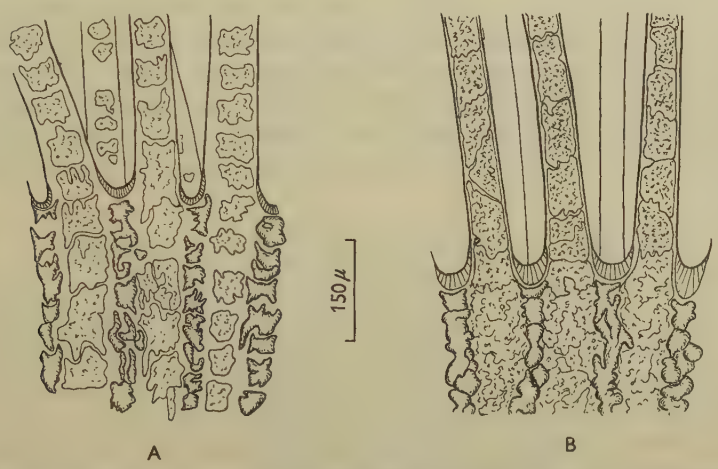

Fig. 9. *Megerlia*: the arrangement of the spicules in the filaments. A. *M. truncata*: a few filaments from the ventral side of a lateral arm of a large specimen, viewed from the abfrontal or outer surface. B. *M. echinata*: a few filaments from the ventral side of a lateral arm of a specimen of shell length 10 mm and width 12·5 mm, viewed abfrontally.

A blood vessel runs within the filamentar canal in the usual frontal position.

Embedded in the supporting substance of the filaments are spicules. These are always present in the outer filaments of the adult where they are large and granulated. The inner filaments are spiculated in some *M. truncata* and not in others, and when present the spicules are small. The spicules of the filaments somewhat resemble those present in the same position in *Platidia*. On the abfrontal surface of the body of the lophophore between the bases of the outer filaments, and in line with the inner filaments, are rows of spicules, which are smaller than those in the basal part of the outer filaments, but are thicker, more rounded, and clear, not granulated (Fig. 9 A).

The distribution of mucous gland cells on the lophophore is similar to that in other plectolophes: a deep abfrontal band below the filaments, paired tracts on the brachial membrane, and scattered mucous cells in the walls of the food groove, including the lip. Mucous glands are especially numerous

on the abfrontal surfaces of both inner and outer filaments, so numerous that they almost touch over the entire surface, although there is a tendency for them to be arranged in two tracts, leaving the mid line clear. Notwithstanding the presence of so many mucous cells, which incidentally obscure the spicules, a good abfrontal current to the tips of the filaments is present. It is possible that the glands have small apertures, for cilia, about 20 μ long, are present over the entire abfrontal surface, although perhaps more closely set on the mid-tract. Mucous gland cells are also frequent on the frontal surfaces of both inner and outer filaments.

The cilia of the filaments are arranged in four tracts; frontal, abfrontal and paired lateral. The dexioplectic wave of the laterals is obvious.

The ciliary feeding mechanism is essentially similar to that already described in *Macandrevia cranium* (Müller) by Atkins (1956), except for some slight differences in the direction of the mantle currents due to the greater width of the valves. It is intended to write a comprehensive account of feeding in brachiopods with plectolophous lophophores, when these differences will be noted.

MEGERLIA ECHINATA (FISCHER & OEHLERT)

A number of *Megerlia echinata* (Fischer & Oehlert) of shell length 1·2 mm and width 1·3 mm to shell length 13·5 mm and width 18·5 mm were dredged by R.V. 'Sarsia' in May 1958 in positions 47° 25′ N., 6° 30′ W., at a depth of 320–490 fathoms; and 47° 57′ N., 7° 54′ W., at a depth of 390–350 fathoms. In the first position they were attached in fair numbers to the dead part of probably growing coral, *Lophelia prolifera* (L.), in company with *Platidia anomioides* (Philippi), *Megathyris detruncata* (Gmelin) and *Crania anomala* (Müller); the latter were mostly cream coloured. In the second position five *Megerlia echinata* were taken, three on coral and two on a large oyster-like valve; one of those on the shell was the largest seen, being 13·5 mm long and 18·5 mm wide. During its first 2 days in the laboratory it gaped naturally some 5 or 6 mm in front; the filaments, however, did not extend. After the second day it remained closed, but at intervals lifted itself some 3 mm in front from the shell to which it was attached. It was dead in a few days. This specimen is considerably larger than the maximum size of length 9 mm and width 12 mm recorded by Fischer & Oehlert (1891) and 12 mm by 16 mm recorded by Massey (1925).

In July 1959 in position 47° 37′ N., 7° 28′ W., at a depth of 780 fathoms three *M. echinata* were dredged attached to an oyster-like valve.

Altogether some 120 *M. echinata* have been dredged. Those on the oyster-like valves were regularly transverse oval, while those on *Lophelia* were frequently irregular in shape owing to crowding by the coral polyps. Some occupied the empty coral cups (Atkins, 1961 *a*). Unlike *M. echinata*, *Platidia anomioides* (Philippi) growing on the same coral and with the same habit,

were generally regular in shape (Atkins, 1959*a*); this was no doubt because of their small size, with maximum length 4·6 mm.

Carmine pigment spots present in *M. truncata* are absent in the deep water *M. echinata*.

A single *M. echinata* of those dredged in May 1958 survived in the tanks to June 1960. A number were alive in September 1959, but most were dead by December 1959. *M. echinata* proved less difficult to relax than *M. truncata*.

Growth stages of the lophophore and loop

A fuller series of growth stages of the lophophore and loop were obtained in *M. echinata* than in *M. truncata*.

At a shell length of 1·4 mm and width of 1·3 mm the late trocholophous lophophore has 16 pairs of long filaments in single series. Both crura and septum are absent. At the same length but with a width of 1·6 mm the circle of the lophophore is slightly interrupted in the anterior mid-line; a septum is still absent. A few more filaments are present than in the preceding stage,

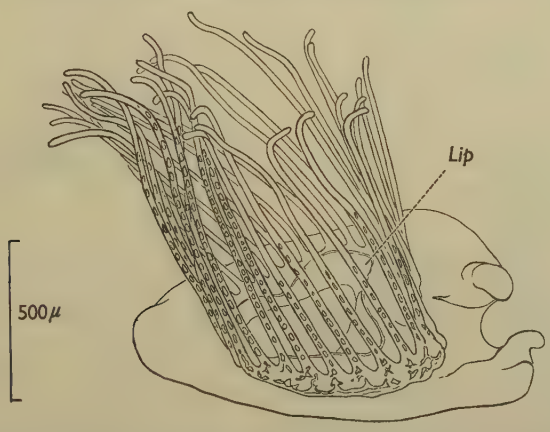

Fig. 10. *Megerlia echinata* of shell length 1·4 mm and width 1·6 mm. Side view of brachial valve with very early schizolophe, narcotized and fixed in formalin. Spicules indicated except in the filaments of the far side. *Lip*, lip of the food groove.

and two outer grooved filaments have appeared on each side. As in the smaller specimen spicules occur at the base of the lophophore, except at the growth region. They are also present in the filaments, extending much farther along the anterior than the posterior ones. The filaments are considerably longer than in the same stage of *M. truncata*; their length may be judged from Fig. 10. In this specimen a small crus is present on one side: tubercles are lacking on the inner surface of the valves.

At a shell length of 2·15 mm and width of 2·4 mm the lophophore is very early schizolophous. A septum has not yet appeared; crural bases only are distinguishable. A few tubercles occur on the inner surface of the valves.

When a shell length of 2·6 mm and width of 2·7–3·2 mm has been attained the lophophore is early schizolophous with a low septum, on the anterior end of which is a hood (Fig. 11 C). At the same length, but with a width of 3·6 mm, short crura are present and the hood is wider (Fig. 11 A, B). In different individuals the septum varies in shape and length, as does also the hood (Fig. 11 A–D).

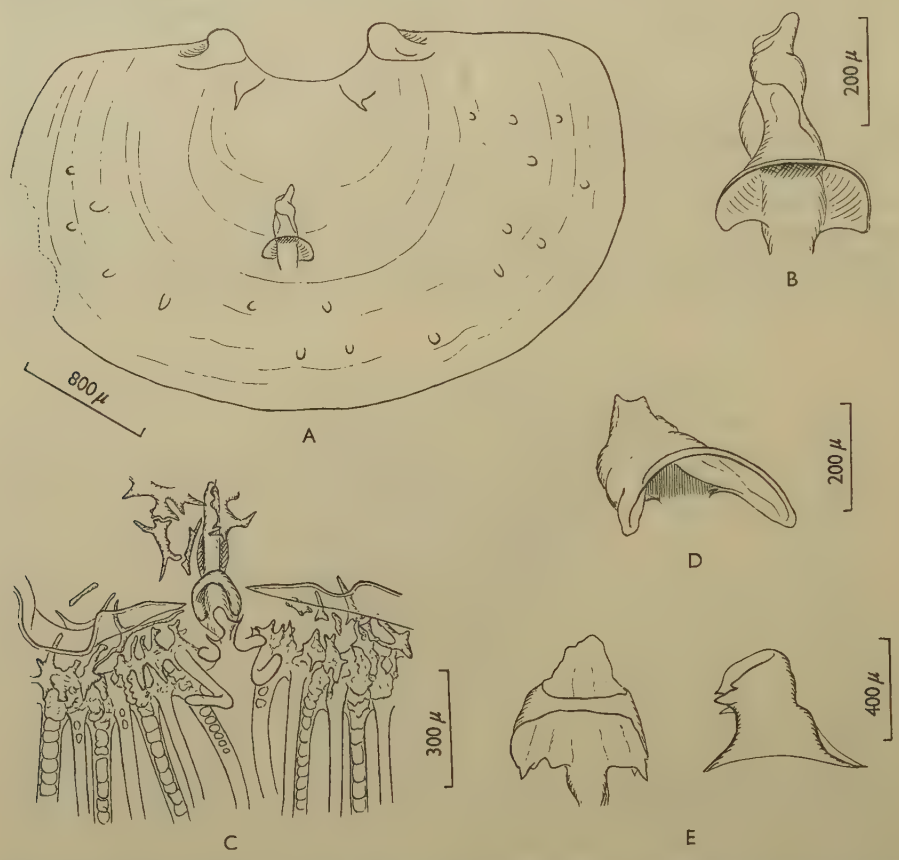

Fig. 11. *Megerlia echinata.* A. Ventral view of brachial valve of an individual of shell length 2·6 mm and width 3·6 mm. B, Septum and hood enlarged. C. Anterior region of early schizolophe of individual of shell length 2·6 mm and width 3·2 mm. D. Septum and hood of individual of length 3·1 mm and width 3·0 mm. E. Septum and hood in ventral and side view of a specimen of length 3·6 mm and width 4·7 mm.

The lophophore is schizolophous at a shell length of 2·9 mm and width of 3·7 mm. The posterior end of the hood has undergone resorption and there is now a transverse band, while the anterior edges of the hood bear spines. The septum and hood of the same lophophore stage are illustrated from a specimen of shell length 3·6 mm and width 4·7 mm (Fig. 11 E).

The lophophore is late schizolophous at a shell length of 4·2 mm and width of 5·2 mm. The brachial support is about as advanced as that shown in Fig. 13 of a specimen of shell length 6·2 mm and width 7·4 mm. Descending branches are beginning to grow from the crura. In an irregularly shaped specimen of shell length 6·0 mm and width 6·9 mm, with a somewht more advanced lophophore, the lateral arms being deflected, the loop is in the stage depicted of a specimen of length 4·5 mm and width 8·0 mm (Fig. 12), but the crura are less developed.

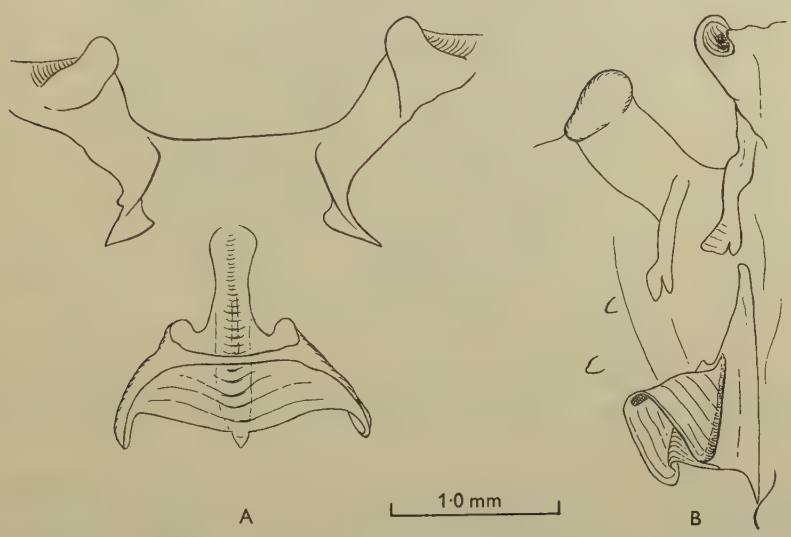

Fig. 12. *Megerlia echinata* of shell length 4·5 mm and width 8·0 mm. Brachidium in (A) ventral and (B) side view.

In another irregularly shaped *M. echinata* of shell length 6·1 mm and width 7·2 mm the lophophore is early zygolophous, with the side arms still only slightly deflected. The anterior extensions of the ring have come to point outwards and backwards. The position of the transverse band to the growth zones of the lophophore is similar to that shown in Fig. 5 of *M. truncata* at a shell length of 4·3 mm and width of 5·6 mm.

The zygolophous stage with side arms deflected is found in a specimen of shell length 6·2 mm and width 7·4 mm (Fig. 13). The brachial support is surprisingly retarded in development for the stage of lophophore.

It will be seen from the foregoing that there is sometimes little change in the brachial support between the late schizolophe with lateral arms only slightly deflected to the zygolophe with lateral arms entirely deflected, but in some individuals at the later stage of lophophore development the support may be as advanced as in that of the early plectolophe. In the specimen of shell length 4·5 mm and width 8·0 mm (Fig. 12) the transverse band is some-

what wider from left to right than in that of shell length 6·2 mm and width 7·4 mm (Fig. 13), and the two divergent processes project well forward. In both, crural processes are present and the beginnings of the descending branches.

Fig. 13. *Megerlia echinata* of shell length 6·2 mm and width 7·4 mm. Brachial valve with zygolophe, narcotized and fixed in formalin. Filaments of the dorsal side of lateral arms omitted. Mucous glands at base of lip indicated.

There is considerable variation in the shell size at which a certain stage of lophophore and loop is attained. In one *M. echinata* of shell length 5·4 mm and width 6·6 mm the lophophore is early plectolophous with one turn to the spiral arm. This seems to be an unusually small shell size for this stage of lophophore. The loop is as in the specimen of shell length 6·2 mm and width 8·7 mm illustrated in Fig. 14. The posterior portions of the descending branches have begun to grow from the crura, while the anterior portions have begun to grow backwards from the antero-lateral divergent processes, which apparently represent the anterior portions of both ascending and descending branches (see Elliott, 1949, p. 542, on *Kraussina*).

The lophophore can be fully plectolophous from a shell length of 9 mm and width of 11 mm (Fig. 15). The filaments are in an inner and outer alternating series, except for some sixteen pairs behind the mouth, which are in single series and have ridged frontal surfaces. The adult loop of a specimen of shell length 12·0 mm and width 15·6 mm is shown in Fig. 16. The descending branches are complete. On one side lacunae have been formed by resorption between the descending and ascending portions of the loop. The median septum now extends as a low ridge almost to the cardinal margin. The outer filaments are heavily spiculated, the inner slightly. At the base of the filaments the granulated and the clear spicules are crowded (Fig. 9B).

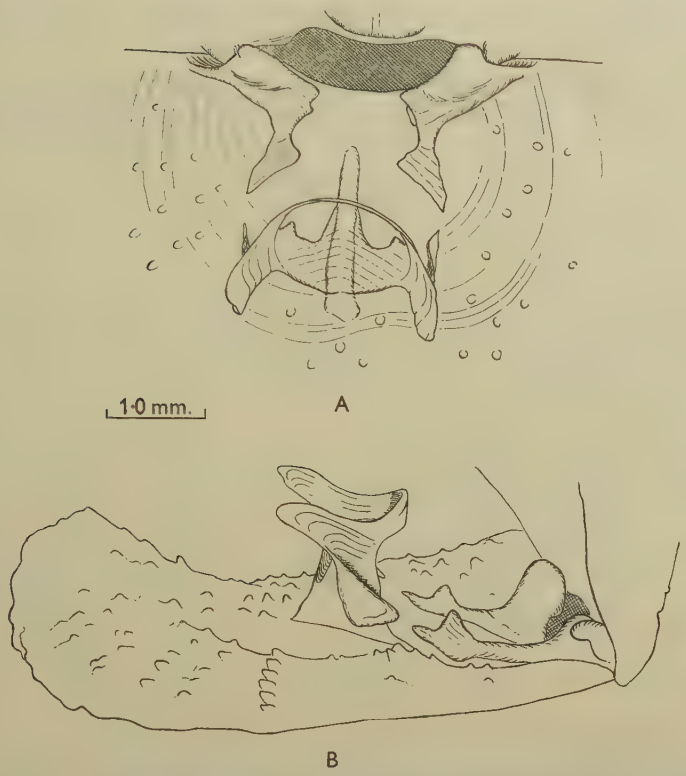

Fig. 14. *Megerlia echinata* of shell length 6·2 mm and width 8·7 mm. Brachidium in (A) ventral and (B) side view.,

Fig. 15. *Megerlia echinata* of shell length 9 mm and width 11 mm. Brachial valve with plectolophe.

In *M. echinata* the filaments in all growth stages of the lophophore are longer and somewhat less wide than in *M. truncata*, as may be seen by a comparison of the figures of the two species. *M. truncata* was dredged by R.V. 'Sarsia' from a depth of 45–48 fathoms, while *M. echinata* came from a depth of 320–780 fathoms. Thomson (1927) gave the maximum depth for *M. truncata* as 303 fathoms, but in at least some instances *M. echinata* has been recorded as *M. truncata*. Some relation may exist between length of

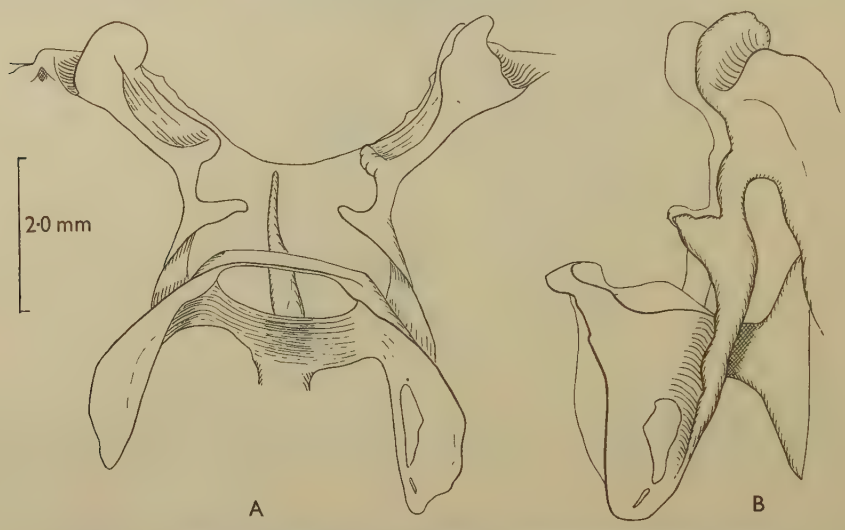

Fig. 16. *Megerlia echinata* of shell length 12 mm and width 15·6 mm. Adult brachidium (A) ventral and (B) side view.

filament and depth at which the species of a genus occurs. In *Platidia*, the filaments of *P. anomioides* occurring with *M. echinata* at a depth of 320–780 fathoms, are considerably longer than those of *P. davidsoni* from a depth of 45–220 fathoms (Atkins, 1959*a*). But more work needs to be done on this. The shell of *M. echinata* is not as thick as that of *M. truncata*.

My thanks are due to the captain and crew of R.V. 'Sarsia' who dredged the brachiopods, and to those members of the staff who were in charge of the scientific work during the cruises. The work was done while occupying a London University Table, and part of it with the aid of a grant from the Brown Research Fund of the Royal Society for the year 1956–57.

SUMMARY

Megerlia truncata and *M. echinata* dredged by R.V. 'Sarsia' have been examined living. The growth stages of the lophophore are described and figured, the series being fuller in *M. echinata* than in *M. truncata*. The

adult lophophore of *M. truncata* has been sectioned and its structure described.

The ciliary feeding mechanism in *Megerlia* is similar to that already described by the author in brachiopods with plectolophous lophophores.

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OBSERVATIONS ON THE MORTALITY OF *OSTREA EDULIS*

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(Text-figs. 1–3)

An account is here given of the mortality rates recorded amongst stocks of oysters at the Tal-y-foel oysterage. This oyster ground, on the Anglesey shore of the Menai Straits (Walne, 1958), is stocked mainly with oysters bred in the Mussel Purification Tanks at Conway. It has therefore been possible to make observations on a stock of homogeneous origin and on separate year groups.

The larvae settle on earthenware tiles, coated with a lime mortar, which are placed in the breeding tanks during the summer and are then transplanted in the late summer or early autumn to wooden racks at the low water mark of spring tides at the oysterage. During the following summer, when the spat measure 1–3 cm in diameter, they are stripped off the tiles and put in wire trays. For studies on growth and mortality populations have been maintained in the trays for many years.

POSSIBLE MORTALITY DUE TO TRANSFERENCE FROM THE BREEDING TANKS TO THE SEA

Two experiments were made to determine the mortality of very young spat in the 2 or 3 months immediately after transplantation from the tanks to the sea, and to compare it with that of spat remaining in the Conway tanks. Because the rough surface of the tiles makes the accurate counting of very small spat difficult, smooth white unglazed saucers, 5 in. (approx. 13 cm) in diameter, were used for these experiments. In 1948 four saucers were used, two kept at the Conway tanks and two planted in a frame at the oysterage; in 1951 six saucers were used, three of which were planted at the oysterage. In both years the maximum age of the spat at the time of planting was 28 days and the mean age about 14 days.

The surviving spat at both the oysterage and at Conway were counted at about monthly intervals and at about the same time. The percentage mortality in each period in the two places is shown in Fig. 1. These results show that not only was the mortality very heavy in the second and third months after settlement—about 50% per month—but also that it was similar in the tanks and at the oysterage. Moving the spat from the sheltered tanks to the open

sea apparently had no immediate effect on the mortality rate, and it can therefore be assumed that the mortalities recorded on the tiles represent a continuous process, with no striking change occurring at or near the time of transplantation.

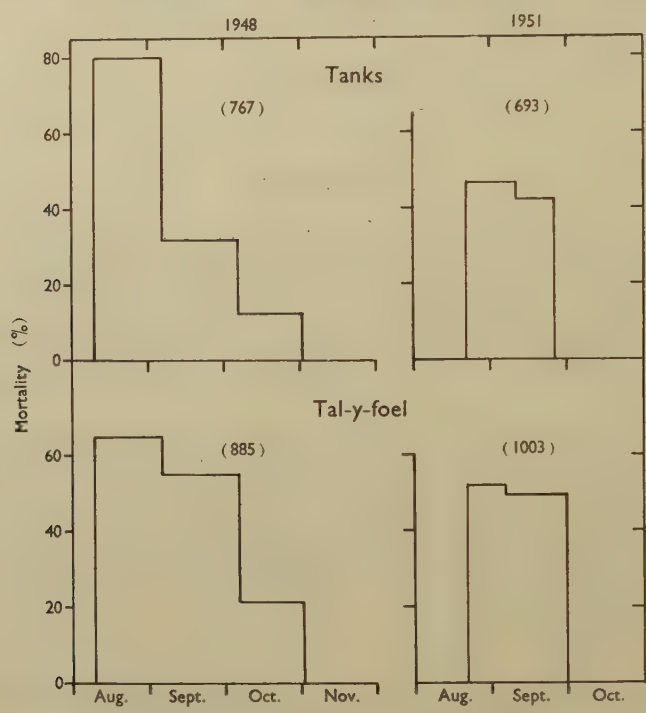

Fig. 1. The mortality of recently settled spat on unglazed saucers kept at the Mussel Tanks and at the oysterage, Tal-y-foel, Menai Straits. The figures in parentheses are the initial numbers of spat.

MORTALITY OF SPAT IN THE FIRST YEAR

The tiles bearing the oyster spat were made up in bundles of ten and taken out to the oysterage in late August or September, when the spat was 1–2 months old. Either at planting, or shortly afterwards, the number of live spat was determined on several bundles of tiles, which were then numbered. The surviving spat were counted the following summer. Mortality observations could not be continued on the tiles any longer, because by this time the lime mortar was becoming rotten and the oysters were becoming detached from the tile.

The observed mortality rate in the first year of life for the six years of observation (Table 1) has varied between 66 and 92%. These data are analysed in more detail below.

Seasonal variations in the mortality rate. Information on the seasonal variation in the mortality rate can be obtained for 4 years in which counts

were made of the surviving spat at intervals during the year. Because the counts were made at irregular intervals the most informative way of treating the data has been to calculate the mean number of spat dying per day during the period, and to calculate this as the percentage of the mean of the number of spat alive at the beginning and end of it. This figure gives the mean daily mortality rate for the period if it is constant throughout, which is unlikely for longer intervals, but is probably reasonably true for shorter ones.

TABLE 1. THE MORTALITY OF SPAT ON TILES PLANTED AT THE TAL-Y-FOEL OYSTERAGE ONE TO TWO MONTHS AFTER THE SPAT HAD SETTLED

Period	No. of tiles	Side of tile	Mean spat per side	Mean % mortality
22. x. 45–13. viii. 46	20	Upper	27·7	91·1
		Lower	159·9	91·9
5. ix. 49–1. viii. 50	80	Upper	4·7	71·2
		Lower	15·6	75·4
14. viii. 50–9. viii. 51	90	Upper	11·8	93·8
		Lower	76·5	89·1
5. ix. 51–11. viii. 52	40	Upper	7·5	69·7
		Lower	80·7	81·6
2. ix. 52–29. vii. 53	50	Upper	1·2	45·7
		Lower	10·3	66·7
25. viii. 53–20. vii. 54	90	Upper	16·0	86·4
		Lower	55·2	90·6

In Fig. 2 the mean daily mortality rate has been plotted against the mid-date of the period for which it has been calculated. To complete the picture the data for the survival of the young spat on the saucers (Fig. 1) has been similarly treated and is also plotted on Fig. 2.

From this figure it appears that the mortality rate declines during the autumn, and remains at a low level from November to March, after which it rises again, but does not, in general, reach such high levels as in the first 2–3 months after settlement. The period of low mortality is a period of low temperature and no growth, but whether the reduced mortality is a reflexion of the reduced activity of the oyster or the reduced activity of possible predators and parasites, or slower growth of competing organisms, is not known.

The effect of the density of spat on the mortality rate. The influence of density on the mortality rate has been difficult to determine and this is perhaps an indication that it is not very important at the densities encountered.

In an experiment in 1950, twenty tiles were planted individually on a special rack at the end of August, and removed for counting on 8 December. The initial numbers of live spat on the tiles ranged from 82 to 224. On the average 57·6% of the spat survived, and there was no tendency for the tiles with the most spat to have a higher proportion of deaths than the more sparsely populated ones.

Some data on mortality and population density are available from the counts made of bundles of tiles, although there are two difficulties in using these data. These are that in any one year the tiles tended to have a rather uniform number of spat, and, secondly, that when the tiles were counted, although they were replaced in the same order, the individuals of the pair at each level were not distinguished. It has therefore been necessary to select from the data pairs of tiles carrying a rather similar population.

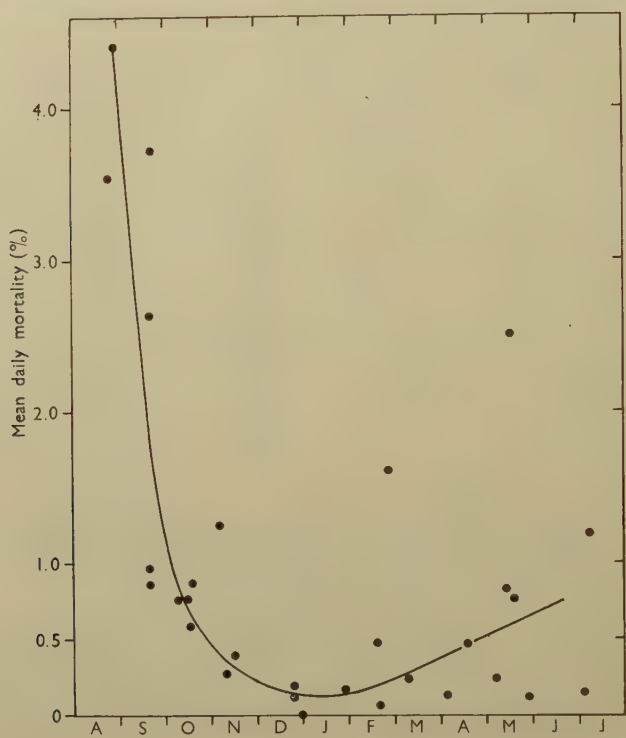

Fig. 2. The mean daily mortality of oysters at the Tal-y-foel oysterage during the year after metamorphosis. Each point is plotted against the middle of the period which it covers. The majority of the spat settled in July. The trend line has been fitted by eye.

Suitable data were found for the years 1950, 1951 and 1953, and for each of these years each pair of tiles with a similar population density was assigned to a high or low initial population group (Table 2) and the mortality rate of the two groups compared for each year. In 1950 there was no significant difference between the two groups, but in 1951 and 1953 there was a significantly ($P > 0{\cdot}01$) higher mortality rate in the higher density groups, although the difference of the means was only 4–5%.

Apparently the under surface of a tile can carry 1 to 250 spat with little effect on the mortality. The under surface has an area of about 480 cm^2, and

if it were completely occupied it would hold 600 spat of 1 cm diameter, 270 spat of 1·5 cm and 150 of 2·0 cm. Since they are not regularly dispersed over the surface and are roughly circular in shape, the oyster spat in a population of 100 will be under an appreciable degree of interference from their neighbours once they reach about 1·5 cm in diameter (the average size during the first winter), but this interference does not seem to be sufficient to cause a severe mortality.

TABLE 2. MORTALITY OF SPAT ON THE UNDERSIDE OF TILES PLANTED AT THE MENAI STRAITS OYSTERAGE FOR ABOUT 10 MONTHS

Pairs of tiles in a bundle have been assigned to one of two groups according to their initial spat density, and the mortality in the two groups compared.

Year	No. of pairs of tiles	Initial spat density		Mean % mortality	Significance of difference
		Range	Mean		
1950–51	23	50–150	116	89·0	None
	21	151–250	187	89·8	
1951–52	10	0–100	61	80·7	> 0·01
	9	200–300	251	85·1	
1953–54	26	50–100	82	88·2	> 0·01
	18	101–250	138	92·7	

Mortality on upper and lower surfaces of the tiles. In four out of six of the yearly records (Table 1) the mortality was greater on the underside of the tiles. Although more spat settle on the underside, the increased mortality is probably due to the heavier settlement of sedentary organisms, barnacles and colonial ascidians in particular, which have a considerable smothering effect on the small spat. The top of the uppermost pair of tiles in a bundle usually has at times during the winter a thick covering of silt, and in the late spring a luxuriant growth of green weed, but the tops of the other tiles are largely free from silt, weed and sedentary organisms.

Mortality due to crabs. As the common shore crab, *Carcinus maenas*, appeared to be the only large potential predator, observations were made at the oysterage on the mortality of spat on tile which were kept in a cage of half-inch wire netting which excluded all except the smallest crabs. The experiment was started with nine bundles of tiles in September 1949 and continued until 1 August 1950. By this time some holes had appeared in the wire netting, so that the oysters were not protected for the latter part of the season, but they were protected for the first few months when they were small. The results were:

	Initial number of spat	Percentage mortality in 11 months
Protected from crabs	885	74·8
Control	1227	73·2

It does not seem from this result that *Carcinus* ate many of the young spat in the first few months after planting.

MORTALITY OF OYSTERS AFTER THEIR REMOVAL FROM THE TILES

A large amount of data has been accumulated on the mortality rate of oysters in trays, but less on those on the ground. The information on those in trays was obtained during studies on growth.

One experiment was made in the period 1952–55 to observe the mortality of two year classes of Conway oysters when laid on the mud at low water mark of spring tides. Two pens, each 1 m square, were made by fixing a wire netting fence between posts at the corners. The fence was 12 in. (30·5 cm) high and was sunk about 3 in. (7·5 cm) below the surface of the mud. Each pen was stocked with 100 oysters, and the number of live and dead was counted at intervals (Table 3). It was found that some oysters

TABLE 3. THE MORTALITY RATE OF TWO YEAR CLASSES OF CONWAY BRED OYSTERS RECORDED OVER SEVERAL SUCCESSIVE TWELVE MONTH PERIODS

The oysters were laid on the mud at low water mark of spring tides.

Year class	Period of observation	Initial no. of oysters	% no. Died	% no. Missing
1948	24. iv. 52–15. iv. 53	100	37	6
	15. iv. 53– 2. iv. 54	57	7	7
	2. iv. 54–25. iii. 55	44	11	0
1950	15. iv. 53– 2. iv. 54	97	11	15
	2. iv. 54–25. iii. 55	71	17	0

completely disappeared from the pens, presumably because of either animal or human interference. The mortalities which are recorded in Table 3 have therefore been calculated from the number of live oysters known to be present at the beginning of each period, and the number of dead shells recovered at the end. The mortalities may therefore have been higher than those recorded in the table. For the 1948-year class the mean mortality over the fourth to seventh year was 16% per year, and for the 1950-year class it was 15% in the third and fourth years.

The wooden trays in which the mortality observations were made measured 5 ft. 4 in. by 3 ft. 0 in. by 4 in. deep (approx. 1·6 m × 0·9 m × 10 cm). The bottoms were of half-inch mesh woven wire for the youngest oysters, otherwise 1 in. chain link was used. The trays were raised on posts about 18 in. (45·75 cm) above the mud and were subdivided into three compartments. The oysters were counted and measured each winter, thus giving the number which died in the preceding period of approximately 12 months. The mortality rate of the oysters in trays (Table 4) was lower than that of those on the ground, until they were over 7 years old. By this age the shells were often badly attacked by *Polydora* and this seems to have been the main cause of the rise in death-rate.

The oysters in the trays and on the bottom were not more than a few yards

apart, and were therefore subjected to the same hydrographic conditions and liability to infection by disease. The main differences in their conditions were that those on the bottom were much more liable to smothering by silt, and also they were exposed to large predators, e.g. gulls, crows and large fish. No losses of adult oysters by large predators were observed, but loss by silt seems very possible. It can act either by slight alterations in the shore level, which would cover those oysters that had become embedded in the mud, or, if the oyster was weak for some reason, the clearing mechanism would not be sufficiently vigorous to deal with the clouds of silt disturbed by the waves and tidal currents.

TABLE 4. PERCENTAGE ANNUAL MORTALITY OF TRAY OYSTERS AT TAL-Y-FOEL

Year group	Initial no. of oysters	Age in years				
		1½–2½	2½–3½	3½–4½	4½–5½	5½–6½
1948	20	—	—	0	0	0
1949	11	—	9·1	0	10·0	11·1
1950	16	—	6·3	0	33·0	10·0
1954	176	—	5·1	3·6	9·9	—
1955	148	2·0	2·1	1·5	—	—
1956	514	6·4	5·8	—	—	—
1957	215	2·3	—	—	—	—
Average % mortality per annum		3·6	5·6	1·0	13·2	7·0

		Age in years				
		6½–7½	7½–8½	8½–9½	9½–10½	10½–11½
1948	20	0	0	5·0	15·8	12·5
1949	11	0	25·0	33·0	25·0	—
1950	16	0	0	11·1	—	—
1954	176	—	—	—	—	—
1955	148	—	—	—	—	—
1956	514	—	—	—	—	—
1957	215	—	—	—	—	—
Average % mortality per annum		0	8·3	16·4	20·4	12·5

There was no indication of seasonal mortality amongst the tray oysters, but the number dying was so few that unless it was very marked it could have escaped notice. Waugh (1954) notes that the majority of oysters that are weak do not die until water temperatures begin to rise.

SURVIVAL CURVE FOR TAL-Y-FOEL OYSTERS

Using the data contained in Fig. 2 and Table 4 a survival curve (Fig. 3) has been constructed, starting with 1000 14-day-old spat.

To cover the first 12 months the daily mortality indicated by the curve in Fig. 1 for the middle of each month has been converted to a percentage mortality for the month. This figure has then been used to calculate successively the number of survivors at the end of each of the first 12 months. The survival

curve is continued by the use of the data in Table 4, but as this gives the mortality from winter to winter, it is necessary to bridge the gap between the end of July when the tile data finish and the following winter when the oysters have recovered from any damage which they suffered when removed from the tiles. There is no information to cover this gap, but there is no reason to suppose that the mortality is particularly high, and a mean daily mortality of 0·75 % has been assigned to this period of 3 months.

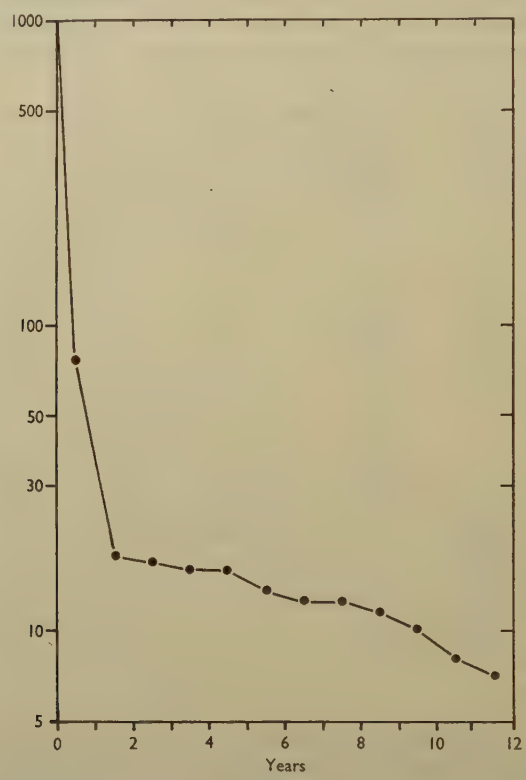

Fig. 3. A survival curve at the Tal-y-foel oysterage for an initial number of 1000 oysters, from metamorphosis until 11½ years old.

This survival curve corresponds with the 'positive skew' rectangular type of Pearl (quoted by Allee, Emerson, Park, Park & Schmidt, 1949), where there is 'an explosive mortality in early life followed by a lingering of the few survivors for a considerable period'. Few examples of this type of survival curve have been worked out, but it has been assumed that marine species with pelagic eggs and larvae would fall into this category (Deevey, 1947). Apparently the oyster has this type of curve even if the pelagic phase is ignored. Of course if this were included the early mortality would be more accentuated.

Once the oysters have entered the fishery at about 18 months old, the mortality is relatively low compared with that of the spat, although it should be remembered that the mortality of the mature oysters recorded in Fig. 3 is low compared with those laid on the ground (Table 3).

DISCUSSION

Records of mortality of *Ostrea edulis* in the literature generally refer to studies of the effect of specific predators or parasites, or to the effect of large-scale disturbance of the habitat: *Urosalpinx* (Cole, 1942; Hancock, 1954), Dutch Shell Disease (Cole, 1950), hard winters (Cole, 1940) and flooding (Waugh, 1954). In the present study all these causes have been either absent or greatly reduced. *Urosalpinx* and Dutch Shell Disease were absent, *Asterias* was very rare. The shore crab *Carcinus* was the most abundant possible large predator, but the experiments reported in this paper do not confirm that it was important. During the period of observation no especially large variation of the weather from the normal occurred.

The mortality rates observed at the Tal-y-foel oysterage may therefore perhaps be described as background mortalities which are similar to those found on other oyster beds, but which are obscured both by the difficulties of observation and by the more obvious deaths caused by predators and large disturbances of the physical environment.

Hancock (1954) estimated that on an oyster ground in the River Crouch 73 % of the spat over 4–5 mm in size died in the period August–December. Because of the difficulty of seeing the recently settled spat in natural conditions, his observations were confined to the larger spat which would have been at least 6–8 weeks old, and therefore any high mortality of the first month or two was not recorded. Of the 73 %, 58 % were eaten by *Urosalpinx* and 15 % died from other causes. Since some of the spat eaten by the *Urosalpinx* would have died anyway later in the experimental period, the mortality from allied causes in a population not preyed upon by *Urosalpinx* would be considerably more than 15 %. For older, mature oysters Orton (1928) estimated their mortality as less than 5 % per annum on the main beds in the Fal.

The cause of death is a matter for speculation and is obviously a potentially fruitful field for research. It is possible that bacteria, protozoa and fungi are always present and ready to invade the weaker specimens. In this context it would appear that the first year is the age when they are particularly vulnerable to attack. Diseases caused by parasitic protozoa in *Crassostrea virginica* have been shown to be of considerable importance (*Dermocystidium*, Mackin, Owen & Collier (1950), and *Nematopsis*, Prytherch (1940), Landau & Galtsoff (1951)), while the presence of *Hexamita* has been confirmed in *Ostrea edulis* (Mackin, Korringa & Hopkins, 1952). The epidemic of unknown origin among *Crassostrea virginica* in Canada is noteworthy (Needler, 1947) and,

as Cole (1951) has pointed out, the very extensive mortalities of the 1919–20 epidemic are still clearly remembered by the industry and it may well be that the causative organism is always present in a mild form.

SUMMARY

An account is given of the mortality of *Ostrea edulis* at the Tal-y-foel oysterage from 14 days to $11\frac{1}{2}$ years after metamorphosis. In the first year after settlement most of the deaths occur in the late summer, autumn and spring, and few during November–February. A survival curve has been drawn up and shown to correspond with Pearl's 'positive skew rectangular type'. On the average, from 1000 14-day-old spat, 78 survive for 6 months, 18 for 18 months and 7 for $11\frac{1}{2}$ years. At this oysterage the major predators are rare or absent, and the cause of most of the mortality is unknown.

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A NOTE ON THE INTERTIDAL LICHEN *LICHINA PYGMAEA* AG.

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(Text-fig. 1)

The shore distribution of *Lichina pygmaea* is now quite well known for numerous localities in the British Isles. The ecology of the lichen in the Plymouth neighbourhood has been described by Naylor (1930), Colman (1933), Evans (1947), and Southward & Orton (1954). There is little information on other aspects of its biology, although Colman (1940) reported on the large animal population found in tufts of *Lichina*. The observations to be described here were made over a number of years on *Lichina* plants growing in the mid-littoral above M.T.L. on the east-facing side of Church Reef (= Blackstone Rocks) at Wembury, South Devon.

Experimental sites were prepared during the summer and autumn of 1952 and 1953 in order to study recolonization after clearance of the lichen. Areas of up to 150 cm^2 were cleared from within the larger lichen 'mats', and half of the lichen growth removed from small circular patches. A small paint scraper was used to detach the lichen tufts, and the underlying barnacles were removed at the same time.

The cleared areas were colonized in turn by sporadic growths of green algae and by barnacles. The changes observed on one of the sites may be seen in Fig. 1, which shows the site at the start of the experiment (Fig. 1 A, B), and then its appearance after a period of four years (Fig. 1 C). In one instance a limited amount of lichen growth from the borders of a cleared area was observed, but the more striking change was the retrogression in the larger 'mat'. Other changes included a localized increase in the density of the fucoid algae with an accompanying increase in the size of a lichen 'mat' where sheltered by the brown algae, and the growth of some of the smaller *Lichina* patches. In addition, a slight retrogression was observed in some of the *Lichina* growths which were not subjected to experimental treatment. The site described lay on fairly steep sloping rocks facing east. Failure of *Lichina* to colonize cleared areas was also observed on experimental sites prepared at the same time in 'mats' of the lichen growing on north-facing rock surfaces, but where these clearances were made no large-scale retrogression was observed.

Stages in the 'natural' retrogression of *Lichina* are frequently observed on

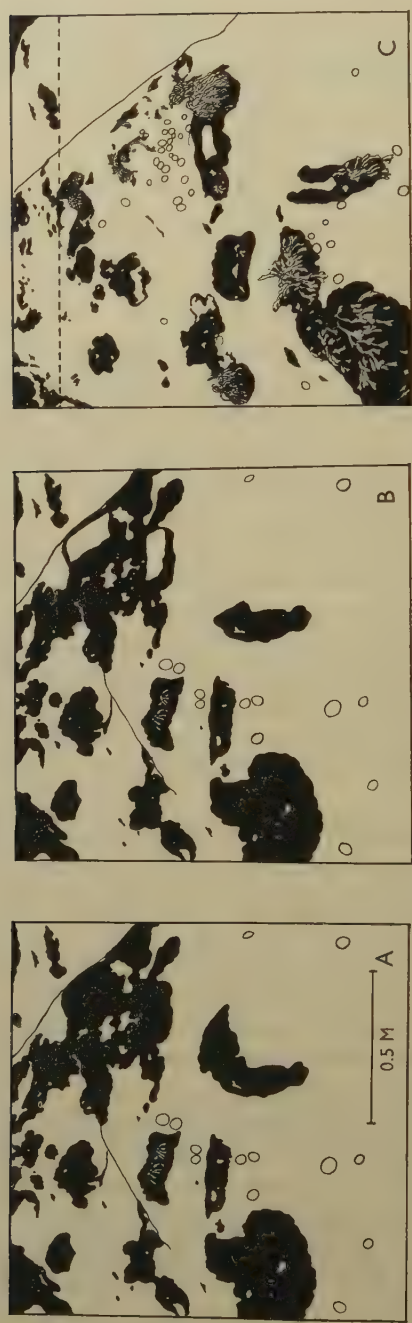

Fig. 1. Diagrams (traced from photographs) of an experimental site at M.T.L. on the east-facing side of Church Reef, Wembury, South Devon. A, Appearance of the site prior to clearance of the lichen (11 September 1953); B, appearance immediately after three clearances of the lichen growth had been made (11 September 1953); C, appearance of the same site 4 years after removal of the lichen growth (13 September 1957). Photographed from a slightly different angle than in A and B. *Lichina* growth shown in black, and the outline of *Patella* shells included; the dense barnacle cover on the rest of the rock surface has been excluded. The broken line at the top of C represents the upper border in A and B.

the shore, and show little relation to the size of the plant growth. The early stages of the process are characterized by a change in branch colour from deep olive-green to orange-yellow, spreading from the branch tips down to the holdfast system. The centre of the 'mat' may show this condition and be surrounded by branches of normal colour. Later stages in the process result in a loss of portions of the 'mat', with stunted yellow-coloured fragments of the plant remaining isolated in the 'cleared' area. Whether this 'die-back' is a result of a particular physiological condition of the plant, or if it is caused by pathogenic organisms with further action by browsing animals, cannot be decided on present evidence. Branch tips which have borne apothecia change to orange-yellow, and some deterioration of the branches may be observed. In this condition the ends of the branches appear similar to those in which the retrogression has reached an advanced state, so that spent fruiting branches may contribute in some way to the early stages in the deterioration of parts of the lichen 'mat'.

It is possible that the act of clearance may have initiated the retrogression of *Lichina* shown in Fig. 1. However, further evidence that this retrogression occurs in the large *Lichina* 'mats' under natural conditions was obtained when the lichen cover of a south-east facing rock was photographed in September 1953, and again in May 1960. Estimates were made of the *Lichina* cover by tracing the outline of the patches of lichen from the photographs, and then measuring the areas of clear rock face and lichen by means of squared paper. In September 1953 *Lichina* covered 41 % of the measured rock surface. In May 1960, several of the smaller *Lichina* 'patches' were still recognizable, but three of the lichen 'mats' showed deterioration. However, four new *Lichina* patches were visible, with the result that the estimated *Lichina* cover in 1960 was 40 % of the measured area. For this small area of the shore there was some degree of stability in the total lichen cover over the seven-year period.

This is one aspect of the ecology of *Lichina* which would repay further investigation, for an analysis of long-term changes would help considerably towards a better understanding of the ecological requirements of the plant. It would be necessary, however, to make observations over longer periods than those used in the present study. Within limited areas of the shore the complex of environmental factors is such that small patches of the lichen may remain unchanged for many years, but a succession of changes in shape and size be shown over the same time by a more luxuriant growth a few centimetres away; and new tufts may appear, or very small tufts show increase in size. It would be of considerable interest to know more about the early stages in the establishment and growth of a lichen tuft, and to know more about the early growth rates of tufts which attain a certain size and then appear to remain unchanged over a number of years.

Evidence has been obtained in the present work which suggests that growth of *Lichina* occurs in habitats where there is some degree of moisture retention

during periods when the rock is not covered by the sea. This growth is indicated in Fig. 1 C; on this particular site protection by fucoid algae resulted in some increase in the area covered by the underlying *Lichina*. The small patches of the lichen which appeared to undergo no change in size over a considerable time interval were situated on rock surfaces above M.H.W.N.T. which drain rapidly. An instance may be given of one small roughly circular *Lichina* patch (diameter approximately 10 cm), which was cleared of half its growth in July 1952; since this time it has remained unchanged in appearance (up to May 1960).

Since growth of the lichen must have occurred in order to establish these small and apparently unchanging patches, the long-term observations envisaged above could yield useful information on growth rates. For example, it is possible that periods of growth may be linked with certain climatic cycles. Similar observations on the plant in different parts of its geographical range would be of interest, for although *Lichina* is found on shores exposed to fairly heavy wave action on the southern coast of the British Isles, Lewis (1957) has described its occurrence on very sheltered and unshaded shingle beaches in west Scotland, and in one instance it was observed mingled with the uppermost plants of *Ascophyllum nodosum* f. *Mackai*.

SUMMARY

Lichina pygmaea failed to recolonize areas cleared from within the lichen 'mats' after four years. A retrogression of the lichen was observed in some instances over periods of 5–7 years. A complex of factors governs the size reached by a lichen tuft; more rapid growth occurs in regions where there is some moisture retention during tidal periods. Although changes occurred with individual 'patches' of lichen, the total *Lichina* cover on a rocky surface remained much the same over a 7-year period.

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THE SPAWNING OF *ARENICOLA MARINA* (L.)

II. SPAWNING UNDER EXPERIMENTAL CONDITIONS

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(Text-figs. 1–2)

This series of papers is concerned with the physiology of spawning and the ripening of the gametes in the lugworm, *Arenicola marina*. Gametogenesis has been described by Newell (1948), and it has been shown (Howie, 1959) that the beginning of the breeding season coincides with, or is preceded by, the first major fall in air temperatures (during low tide) in the autumn. It is suggested that this may provide the external spawning stimulus. In the light of data collected from various localities Duncan (1960) states that 'the close coincidence of breeding with tidal phases indicates that some external factor(s) associated with tides are responsible for the release of the germ cells'. On *a priori* grounds this type of stimulus would appear more likely in the case of a species which spawns at regular intervals than in *Arenicola* which, in general, has a single annual breeding season of about 3 weeks' duration (Newell, 1948; Duncan, 1953, 1960; Howie, 1959). Moreover, examination of the data presented by Duncan and by Howie indicates that the peak of spawning, and more important, the beginning of spawning, is not always associated with a particular phase of the tidal cycle.

Palmer (1937) and Sandeman (1954) have shown that ground-up tissues of *Arbacia punctulata* and *Asterias rubens* when injected into the perivisceral cavities of ripe specimens of these species will induce shedding. This stimulated the work described now in which the possibility was investigated that the spawning of the lugworm might be immediately effected by an agent recoverable from the tissues. The results may also have some bearing on the problem of the external spawning stimulus.

Part of the work described here was carried out at the Gatty Marine Laboratory, St Andrews, where the author was in receipt of a grant in aid of research from the Carnegie Trust. Grateful acknowledgement is made to Dr J. M. Dodd for help and advice, and to Mr E. J. Sandeman who co-operated fully in the earlier part of the work.

INITIAL EXPERIMENTS

As noted above, the vast majority of the lugworms found around the British and Irish coasts, including those found at St Andrews and Dublin, have a restricted breeding season of about 3 weeks' duration occurring during the

autumn. The initial experiments were made about 5 weeks before the breeding season began.

A mixed sample of fifty male and female lugworms was emulsified in a macerator for about 10 min. Each worm yielded about 7·0 ml. emulsion. The emulsion was then lightly centrifuged to bring down the sand present and the mother liquor was decanted off. This was passed through Whatman no. 1 filter-paper and the filtrate (crude extract) was injected into freshly collected worms which had been washed free from sand and placed in large (1 l.) crystallizing basins filled with sea water. Two experiments were carried out with male worms. In the first experiment twenty-four worms were each given 0·5 ml. of the crude extract. Injection was made through the ventral body wall into the coelom; care being taken to avoid damage to the nerve cord and blood vessels. It was found that the puncture sealed with little or no loss of coelomic fluid. All the worms reacted violently to the injection. Some made swimming movements; others exhibited extreme contraction of the body wall so that the tail frequently split between segments with the emission of quantities of coelomic fluid and spermatozoa.

Drops of coelomic fluid containing sperm morulae (sperm plates) were taken from the body cavities of nine worms between half an hour and 1 h after injection. Many sperm morulae were found to have been broken down to free spermatozoa which became intensely active upon dilution in sea water. The coelomic contents of many hundreds of worms have been examined in the course of this work and breakdown of sperm morulae to free spermatozoa in the coelom has been observed only in worms which were actually spawning or which were dying and undergoing histolysis. By approximately 2 h after injection the majority of the worms had relaxed and were showing more normal rhythmic contractions of the body wall. At about this time nine worms began spawning. Shedding appeared normal in every respect. Subsequently it has been discovered that emission of spermatozoa may continue for at least 20 h. The sperm exuded was found to consist entirely of free spermatozoa which soon became active in the water, producing active suspensions.

The second experiment with male worms was carried out approximately a week later. Eighteen worms were injected with crude extract and six worms were given 0·5 ml., 0·5 M saline (as controls). In half the worms the volume of the coelomic fluid was reduced prior to injection by an amount equal to the volume of the injection. Fifteen of the worms injected with extract spawned. The average time elapsing between injection and spawning was 89 min. No control worms spawned. As in the first experiment injection of extract immediately produced violent contraction of the body wall and splitting of the tail. This was not merely the effect of increased pressure in the body cavity. Control worms showed little reaction to injection of saline other than a brief contraction. Furthermore, among worms given extract, splitting of the tail occurred with the same frequency whether the volume of coelomic fluid had

or had not been reduced prior to the injection of extract. It would appear, then, that a muscle stimulant is present in tissue extracts.

Ten females were injected with crude extract under exactly similar conditions to the experiments with males described above. These worms also exhibited contraction of the body wall but spawning did not occur. Oocytes were taken from the coelom (coelomic eggs) 7 h after the worms were injected with extract, but they showed no change from the normal appearance of coelomic eggs (see Newell, 1948).

These experiments show that injection of crude tissue extract produces a strong spawning reaction in male worms. This occurs after a prolonged latent period of about $1\frac{1}{2}$ h. As the lugworm has a restricted breeding season it would appear unlikely that a number of specimens would spawn spontaneously in the laboratory some 5 weeks before the breeding season is due to commence. This view is supported by the evidence of the controls. These experiments failed to indicate whether the muscle stimulant has some role in the initiation of shedding.

With regard to the experiment with females, it seems possible, in view of Scott's (1911) observations on oviposition in *Amphitrite ornata*, that at an early stage in the season the oocytes might not have attained a sufficient size or density for them to be taken up by the coelomic funnels of the nephromixia and shed. It was decided to postpone further experiments with females until such time as more was known of the spawning stimulant. Experiments on females are still continuing and will be discussed in a subsequent paper.

FRACTIONATION AND ASSAY OF TISSUE EXTRACTS

Male and female worms were macerated and the emulsion treated and extracted in various ways, in an endeavour to isolate the spawning stimulant and discover some of its properties. The various fractions produced were assayed for potency in inducing spawning by injecting them into male worms.

Assay technique

The initial experiments suggested both qualitative and quantitative criteria for assaying the potency of the extracts to be tested.

Qualitative: the percentage of worms which spawned (S) in response to the extract injected.

Quantitative: (1) the time (T) in minutes, elapsing between injection of extract and onset of shedding. (2) the activity (A) of the spermatozoa shed. (3) the degree of spawning (D), i.e. the total quantity of sperm shed. In the assays described below variable quantities of spermatozoa were shed into a large volume of sea water so that the preparation of numerous suspensions of standard density for the objective measurement of relative sperm activity would have proved virtually impossible (see Bosselaar & Spronk, 1952;

Rothschild, 1953). In order that each extract could be assayed upon as many worms as possible, it was decided to rely upon a rapid, subjective measurement of sperm activity. Freshly spawned samples of spermatozoa were examined under the microscope and the activity of each was assessed on an arbitrary scale of 5 units (Table 1). In an experiment in which the activity of a number of samples was estimated and then re-estimated in random order, the error in grading was found to be less than 6%. With regard to criterion *D*, at first measurement depended upon an estimate of the size of sperm puddle shed by each spawner. Again this was assessed on an arbitrary scale of 5 units (Table 1). Subsequently, the relative quantity of sperm shed was measured

TABLE 1. ASSAY OF THE ACTIVITY OF SPAWNED SPERM AND THE DEGREE OF SHEDDING

Assay scale	Activity of spawned sperm	Degree of shedding: Size of sperm puddle*	Degree of shedding: Colorimeter value (log scale)	Degree of shedding: Sperm $\times 10^6$/ml. (in the range)†
0	No activity	Insufficient to form puddle	$<0{\cdot}5$	$<2{\cdot}5$
1	A few spermatozoa show slight activity	Very small puddle	0·5–4·0	2·5–20
2	Poor, but general activity	Poor puddle	4·0–7·0	20–35
3	Good activity, dense suspensions agglutinate	Good puddle	7·0–9·5	35–52·5
4	Very good activity. Rapid agglutination of dense suspensions	Large puddle, suspension obscures worm	9·5–12·5	52·5–67·5
5	Intense activity	May become spent	$>12{\cdot}5$	$>67{\cdot}5$

* After 3 h.

† Overnight; total volume of suspension 800 ml.

by placing each worm in a fixed volume of sea water (800 ml.) and measuring the degree of light absorption of any sperm suspension produced using an 'EEL' portable photoelectric colorimeter (see Tacken, 1952; de Wael, de Bois & Hendrikse, 1952). Worms injected with extract were allowed to lie undisturbed overnight (18–24 h). The shed sperm was then vigorously stirred to produce an even suspension, samples of which were pipetted into standard (10 ml.) tubes and the degree of absorption of light measured. A blue filter (*ca.* 485 mμ) was used as this light was readily absorbed. Thus, relatively dilute suspensions gave an appreciable reading. The machine was zeroed (nil absorption) using sea water as a standard. The colorimeter was calibrated to match as closely as possible the 'pool size' assay scale of 5 units mentioned above and counts were made with a 'Metz' haemocytometer to give the absolute numbers of spermatozoa represented by each grade on the assay scale (Table 1).

The assays have been carried out during the 5–6 weeks preceding the breeding season in the autumn over a number of years. They were set up and the injections were made in the same way as described for the initial experi-

ments with males. As no benefit appeared to be gained by reducing the volume of the coelomic fluid prior to injection, this practice was discontinued. During the first season fifty-two worms were given 0·5 ml., 0·5 M saline as controls. Only three of these spawned, of which two spawned in an experiment carried out after the beginning of the breeding season. Similar results with control worms have been obtained in subsequent seasons. They will not be referred to again except in so far as the appropriate results with control worms are given in tables illustrating the results of assays.

Further experiments with crude extracts

In the majority of experiments during the first season a few worms were given crude extract for purposes of comparison. Sixty-six worms were given the crude extract and sixty-one spawned. In the vast majority shedding began 60–90 min after they were injected. The activity of the spawned sperm was frequently comparable to that spawned naturally, and on average a good quantity of sperm (criterion *D*) was shed (Fig. 1). Although the worms must have increased in ripeness during the period in which the assays were carried out, the results were surprisingly uniform (Table 2). This suggests that it is permissible to compare the results given by extracts assayed at different times during the weeks immediately prior to the breeding season.

TABLE 2. ASSAY OF CRUDE AQUEOUS EXTRACTS

Date	Worms injected	No. spawning (*S*)	Time (*T*) (min)	Activity (*A*)	Degree (*D*)
17. ix.*	18	15	89	4·4	1·7
18. ix.	4	4	101	4·0	3·2
19. ix.	8	8	69	3·9	2·7
24. ix.	6	6	69	4·3	3·3
24. ix.	6	6	78	4·3	3·2
25. ix.	6	6	97	3·7	2·0
1. x.	6	5	69	3·5	2·7
2. x.	4	4	75	4·5	2·7
16. x.	6	5	100	3·2	2·2
17. x.	2	2	?	3·5	2·0
	66	61	83†	4·1†	2·5†
Controls	28	2	—	—	—

* Initial experiment.

† Values of criteria *T*, *A* and *D* given here and in subsequent assay tables are average values only for worms which spawned.

In order to test the spawning stimulant for heat stability crude extracts were boiled for 10 min and kept above 85° C for a further hour. The heat-treated extract was then cooled and administered to ten worms. All these worms spawned and the values for all the assay criteria showed no significant decrease in relation to those with untreated extract (Table 3). There is no doubt that the heated extract remained potent. Six of the ten worms given the heat-treated extract showed breaking of the tail.

Although the crude material can be regarded as an aqueous extract of the whole tissue, it was only paper-filtered, so that the active agent could have been present in suspension. Crude extracts were therefore ultra-filtered through Carlson E.K. asbestos sterilizing filter film and the filtrate was administered to twenty-four worms. After a protracted lapse of time (average, 192 min) four worms spawned. The first sperm spawned by three of these specimens was still united in morulae and the activity of the shed sperm was poor throughout (Table 3, Fig. 1). Subsequently, crude extracts were heated prior to ultra-filtration and a further fourteen worms were given the filtrate with markedly improved results. Twelve worms spawned, but the activity and quantity of the sperm spawned remained poor relative to results with paper-filtered crude extracts (Table 3). In these experiments with ultra-filtered extracts nineteen of the worms showed fragmentation of the tail.

It is apparent from the assays so far described that the spawning stimulant present in crude extracts is heat stable under the conditions of treatment described, and that it is very sparingly soluble in water although the solubility or degree of dispersion is increased by heating. From this it was concluded that the active agent is not a protein. The agent causing fragmentation of the tail was not eliminated by heating or by ultra-filtration of the crude extract.

Solubility in fat solvents.

In view of these results with aqueous extracts, emulsified worms were extracted with ether (see appendix A). After carrying out the extraction, the solvent was separated and either paper-filtered or ultra-filtered before recovery of the solute by evaporation. To guard against the possibility of the active agent being carried in a water fraction, benzene extracts were also prepared in which the emulsion produced by macerating the worms was first dehydrated before extraction with the solvent.

The residue after evaporation of the solvents was a dark brown viscous oil and gave off a strong fish-oil odour. The yield per 0·5 ml. of the original emulsion was calculated (6·5–9·0 mg for worms containing gametes) and this amount made up in 0·5 ml., 0·5 M saline was administered to each worm for assay. Continuous shaking was required to suspend the oil in saline for injection. In recent experiments arachis oil has been used as the carrier in which these extracts were injected. In this case 0·5 ml. arachis oil was administered to control worms and it was found that it did not induce spawning. Thirty-four worms were given paper-filtered ether extracts and twenty-nine worms ultra-filtered ether extracts. Sixty worms were given paper-filtered benzene extracts, and twenty worms ultra-filtered benzene extracts. Of the total of 143 worms, 139 spawned. The assays of ether and benzene extracts compare favourably with results given by crude extracts (Fig. 1). There is some reduction in the potency of the extracts upon ultra-filtration but this is very slight in relation to the effect of ultra-filtering crude

extracts (Table 3). Small quantities of hot acetone extracts were also prepared and assayed (see appendix A). Acetone also proved capable of extracting the spawning stimulant from macerated tissue but upon ultra-filtration the potency of these extracts was much reduced (Table 3).

The spawning stimulant is therefore a lipid, readily soluble in benzene and ether. Although only a small number of worms was used in assaying the

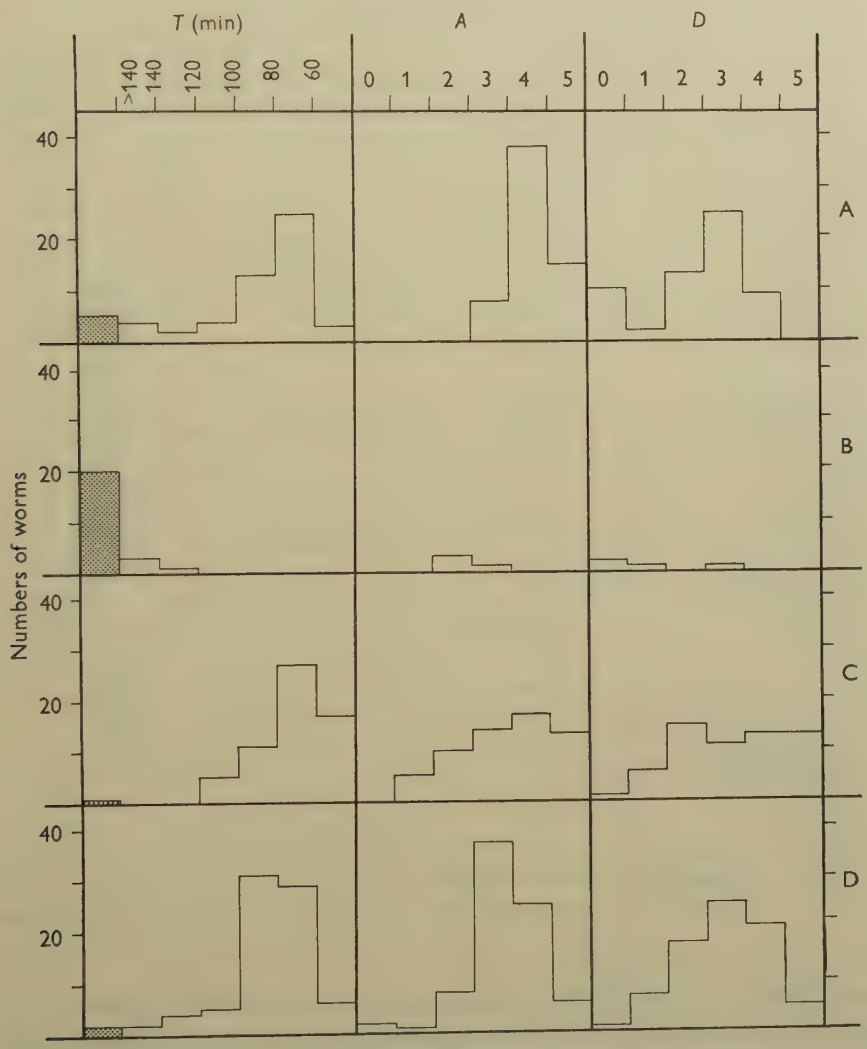

Fig. 1. Responses, measured by various assay criteria, by worms given: A, crude extracts; B, ultra-filtered crude extracts; C, ether extracts; and D, benzene extracts. Stippled areas indicate number of worms failing to spawn in response to the extract injected. The criteria are *T* (time taken to spawn), *A* (activity of sperm), and *D* (degree of spawning).

acetone extracts there seems little doubt that acetone is a poor solvent for the spawning stimulant. During the first season in which this work was carried out, eighty-one worms were given fat-solvent extracts; of these, only nine showed splitting of the tail. The muscle stimulant present in crude extracts is therefore poorly extracted by fat solvents and does not appear to be associated with the action of the spawning stimulant.

TABLE 3. ASSAY OF VARIOUS EXTRACTS

Extract	Assays	Worms injected	Assay criteria: Worms spawning (*S*)	Time (min) (*T*)	Sperm activity (*A*)	Amount spawned (*D*)
Crude						
Paper filtered	10	66	61	83	4·1	2·5
Paper filtered heat treated	2	10	10	94	3·8	2·3
Control		6	0	—	—	—
Ultra-filtered	2	24	4	192	2·0	1·0
Control		6	0	—	—	—
Ultra-filtered and heat treated	3	14	12	78	1·0	1·3
Control		8	0	—	—	—
Ether						
Paper filtered	8	34	34	67	4·0	3·5
Ultra-filtered	8	29	27	68	2·8	2·7
Control		30	1	—	—	—
Benzene						
Paper filtered	9	60	58	81	3·4	2·9
Ultra-filtered	3	20	20	79	3·2	2·6
Control		49	1	—	—	—
Acetone						
Paper filtered	3	20	18	87	2·5	2·1
Ultra-filtered*	1	8	6	76	1·5	1·0
Ultra-filtered†	1	4	3	72	1·3	1·0
Control		15	0	—	—	—
Ether						
Of fatty acids	12	78	69	91	3·0	2·2
Of (unsaponifiable) 'cholesterol fraction'	6	44	3	111	1·3	0
Of (unsaponificable) 'mother liquors' fraction	2	14	0	—	—	—
Control		33	5‡	—	—	—

* Filtrate after ultra-filtration.

† Material re-extracted from asbestos ultra-filter pads.

‡ Four of these spawned in an experiment conducted after the breeding season commenced.

Saponification of the lipid

In preliminary experiments the fats in macerated worm tissues were saponified: (1) by boiling the macerated tissue with an equal volume of 20% sodium hydroxide for 45–60 min; or (2) by boiling the fat obtained after ether extraction with an excess of 20% sodium hydroxide or 10% alcoholic potassium hydroxide for up to 3 h.

When the unsaponifiable fractions obtained by these methods were recovered and injected into male worms, the fraction produced by method 1 (above) gave results comparable with those given by the total lipid but the fraction produced by hydrolysing the extracted fat (method 2) induced little shedding and the spawned spermatozoa were almost inactive. These results suggested that the active principle might be saponifiable under more rigorous hydrolysis.

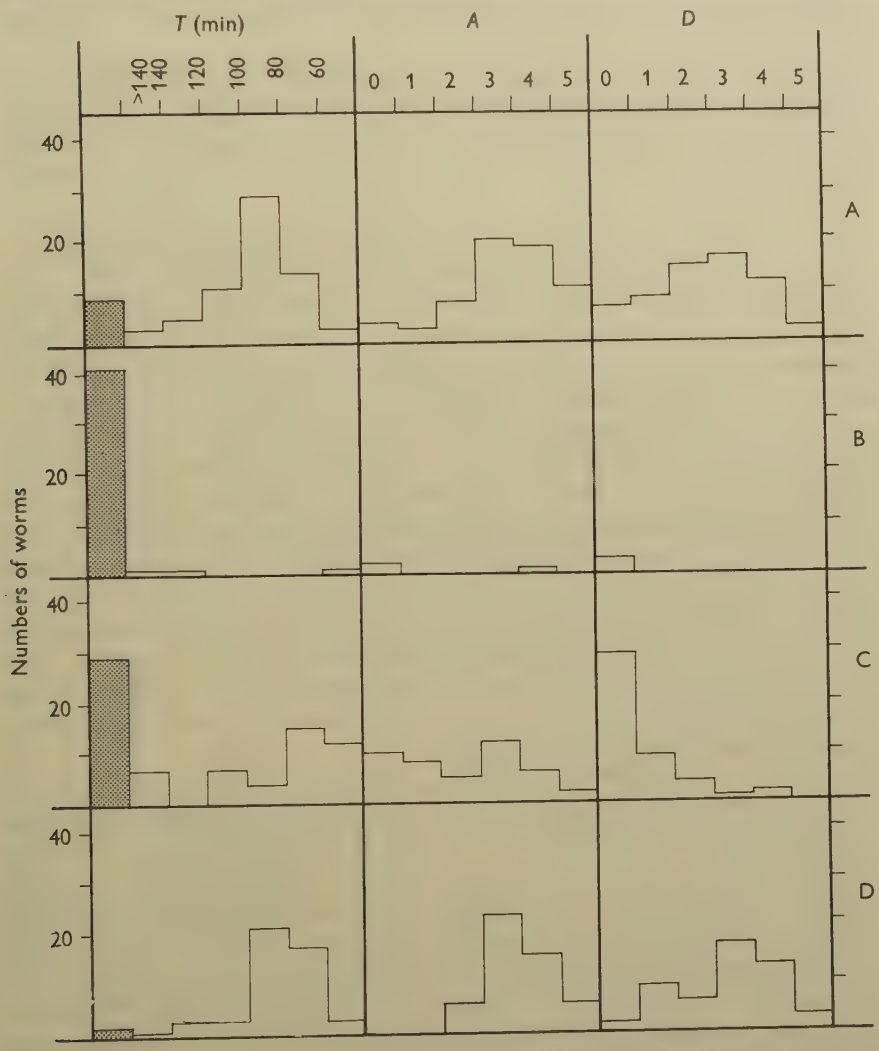

Fig. 2. Responses, measured by various assay criteria (*T*, *A*, *D*), by worms given: A, the fatty acid fraction of tissue extracts; B, the 'cholesterol' fraction of tissue extracts; C, benzene and ether extracts at temperatures above 13° C; and D, benzene and ether extracts at temperatures below 12° C (compare Fig. 1 D). Stippled areas indicate numbers of worms failing to spawn in response to the extract injected.

The author is indebted to Dr T. B. H. McMurry, Chemistry Department, Trinity College, Dublin, for carrying out further saponification of the fat to test this possibility. Saponifiable ('fatty acid') and unsaponifiable ('cholesterol' and 'mother liquor') fractions were recovered (see appendix A). In this case the results of the assays were clear-cut. The fatty acid fraction proved potent in producing spawning, while the unsaponifiable fractions failed to induce shedding in fifty-five of the fifty-eight worms to which they were administered (Table 3, Fig. 2).

Further fractionation of the 'fatty acid' fraction is being carried out in an attempt to discover the molecular species responsible for the shedding reaction. Experiments with a number of known saturated fatty acids indicate that these fail to induce shedding.

THE OCCURRENCE OF THE SPAWNING STIMULANT

Extracts were prepared from male worms and from female worms only. A third extract was prepared from worms containing no genital products. These were presumed to be immature as at the time the majority of the worms contained developing gametes. Upon assay all these extracts proved to be potent in inducing spawning (Table 4). Subsequently ether or benzene extracts were prepared from worms collected at Booterstown, Dublin, during periods when the worms: (1) contained no genital products (February), (2) contained gametocytes only (June), (3) contained oocytes and sperm morulae (September and October), (4) were spawning, a number being spent (November). These extracts were assayed in a purely qualitative manner, i.e. the lipid produced in each case was administered to worms for assay at a fixed concentration (6·5 mg in 0·5 ml. arachis oil/injection) without regard to variation in yield during the year. Again all the extracts proved potent in inducing the spawning of male worms (Table 4). Table 5 gives information on fat yields from extractions carried out at various times of year.

In order to examine the anatomical distribution of the active material a number of worms were dissected and ultra-filtered ether extracts of various body parts were prepared. The nephromixia, with gonads attached (see Ashworth, 1904) were ground up in a mortar and extracted by ether for 3 days. Extracts of the gut plus accessory glands and of the body wall were prepared in the same way. Finally, the coelomic fluid plus genital products were extracted by ether after alternate freezing and thawing. The residues were made up in equal volumes of the carrier, without regard to the variation in yield, it being argued that if the active substance were concentrated in any one region a full reaction would be obtained irrespective of the total fat yield from that region. Each extract was administered to six worms and all induced spawning in at least one worm (Table 4). Extracts of the gut and of the coelomic contents both induced spawning in five out of six worms and the

average quantity of sperm spawned was not much less than that shed in response to the injection of similar extracts of whole worms. Little significance can be attached to the poor sperm activity with the gut extract as it was found in the course of the work with fat-solvent extracts that trace quantities of solvent remaining in the material injected were sufficient to inhibit sperm activity although shedding might not be affected. Traces of sperm were shed

TABLE 4. THE OCCURRENCE OF THE ACTIVE SUBSTANCE

Assays of extracts from : (1) males, females and worms not containing genital products (NGP); (2) various body parts (ultra-filtered ether extracts); (3) worms collected at various times of year (benzene extracts).

	Extract of	Worms injected	Worms spawning (*S*)	Time (min) (*T*)	Sperm activity (*A*)	Amount spawned (*D*)
(1)	Males (crude extract)	6	5	72	4·0	3·0
	Females (crude extract)	6	6	78	3·7	2·7
	Worms NGP (ether ultra-filtered)	6	6	55	2·7	2·3
(2)	Body fluid and gametes	6	5	73	3·6	2·4
	Gut	6	5	58	1·2	2·2
	Nephromixia	6	2	134	3·0	0
	Body wall	6	1	36	3·0*	3·0*
(3)	Worms collected during:					
	February	6	6	<91	3·7	3·7
	June	6	5	91	4·6	3·0
	September	9	8	<109	3·6	2·6
	October	10	8	<101	4·0	2·3
	November	8	7	<102	3·0	2·9
	Control	8	1	—	—	—

* Figures for one worm only.

TABLE 5. FAT YIELDS FROM WORMS COLLECTED AT BOOTERSTOWN, DUBLIN

Date	No. of worms	Gametes	♂	♀	N.G.P.	Av. wet weight (*G*)	Total fat (*G*)	Fat/worm (*MG*)	Solvent
ii. 58	62	Nil	0	0	62	—	1·69	27	Benzene
vi. 58	85	Gametocytes only	—	—	—	—	2·04	24	Benzene
vii. 59	100	Eggs and sperm	—	—	—	11·86	13·11	131*	Benzene
ix. 55	52	Eggs and sperm	27	18	7	—	6·03	116	Ether
ix. 58	119	Eggs and sperm	69	48	2	9·84	11·90	100*	Benzene
x. 55	57	Eggs and sperm	32	22	3	—	6·44	113	Ether
x. 55	52	Eggs and sperm	10	38	4	—	6·34	122	Ether
x. 57	68	Eggs and sperm	36	30	2	—	8·76	129	Benzene
xi. 57	34	Half worms spent	6	8	20	—	1·77	52	Benzene

* Percentage wet weight fat, 1·1 and 1·02 respectively.

by two worms which were given the extract of nephromixia. The total fat yield from this tissue was small but it is apparent that it contains some of the active substance. The body-wall extract was administered for assay in relatively high concentration due to the fact that this region contains a high total of fat in relation to the gut (see Dales, 1957). However, the body-wall

extract induced spawning in only one of the six worms to which it was administered. A simple dilution experiment with crude extract shows that a 1:1 dilution of the normal amount of crude extract injected has little effect upon the spawning reaction, but in greater dilutions (Table 6) the reaction in respect of number of worms spawning and amount of sperm spawned falls off sharply although the traces of sperm shed may be active. While no great stress can be laid on a single assay with a small number of worms the body-wall fraction appears to contain only a small amount of the active substance as compared to the gut and the coelomic contents.

TABLE 6. THE EFFECT OF DILUTION OF A CRUDE EXTRACT

Parts extract	Parts saline	Worms injected	Worms spawning (*S*)	Time (min) (*T*)	Sperm activity (*A*)	Amount spawned (*D*)
1	0	6	5	69	4·2	3·2
1	1	6	5	59	4·0	2·6
1	5	6	4	81	3·7	0·7
1	10	6	2	110	4·0	0
1	500	6	—	—	—	—
Control		4	—	—	—	—

THE EFFECT OF TEMPERATURE

The first assays in each season were frequently variable in result, even when the same extract was administered, and during two seasons in Dublin the results were variable throughout. At this time the work on the environmental spawning stimulus, referred to above (Howie, 1959), indicated that on the shore there might be a threshold limit of temperature above which spawning will not take place. It was noted that during the two seasons when unsatisfactory results were obtained the experiments were carried out in a heated laboratory. In view of these observations an attempt was made to determine the influence of temperature upon stimulation of spawning in the laboratory.

Experiments were carried out in which extracts were administered to male worms kept in an out-house so that the water temperature was approximately the same as air temperatures for the time of year. In further experiments groups of worms were adjusted to differing temperatures overnight, and in one case over a period of 3 days, and then injected simultaneously with extract. A record was also kept of the average degree of maturity of the worms used. In the case of males increasing maturity is indicated by an increase of the percentage of coelomic sperm cells bound in morulae.

The results of the assays (Table 7) show no correlation with the degree of maturity of the worms even at an early stage in the season. Thus on 16 October 1958 a full spawning reaction was induced by tissue extracts when the morulae count was only 54%, whereas poor or variable results were obtained on 20 and 28 October when sperm counts revealed 54–67% of the sperm cells in morulae.

Seventy-three worms were given tissue extracts in experiments in which the water temperature exceeded 13·5° C (maximum 15·5° C). Of these, twenty-nine failed to spawn and only fifteen shed a measurable quantity of spermatozoa. In the range 12·1–13·1° C, sixteen out of the seventeen worms given extract spawned; the quantity and activity of the sperm shed was variable and on average somewhat less than the uniformly high assay results which were produced by extracts at temperatures below 12° C (Table 7, Fig. 2). An exception occurred in the experiment in which worms were acclimatized for 3 days to approximately 7·5° C, when poor results were

TABLE 7. INFLUENCE OF TEMPERATURE AND DEGREE OF MATURITY UPON THE RESPONSE OF MALE WORMS TO THE INJECTION OF TISSUE EXTRACTS (ETHER OR BENZENE)

Date	Sperm in morulae (%)	Water temp. (° C)	Worms injected	Worms spawning (*S*)	(*T*)	(*A*)	(*D*)
22. ix. 58	44	15·5	17	10	77	0·7	0
(1) 28. x. 59	66–83	15·5	8	5	76	3·2	0·8
9. x. 57	57	14·7	15	13	69	2·3	0·7
20. x. 58*	54	14·4	7	4	>160	1·2	1·2
7. x. 58	52	13·8	16	11	82	2·6	0·7
(3) 13. xi. 59	83	13·5–14·5	10	1	50	0	0
(2) 21. x. 59	60–66	12·9–13·1	7	6	79	3·0	1·3
28. x. 58	67	12·5	4	4	108	2·5	1·5
3. xi. 58	?	12·1	6	6	58	2·5	2·7
22/29. x. 57	64–75	11·6–11·8	11	11	58	3·9	3·2
16. x. 58	54	8·8–11·8	21	21	86	3·1	2·5
(2) 21. x. 59	60–66	8·2–8·4	7	6	84	3·5	2·7
(1) 28. x. 59	66–83	7·0–8·0	8	8	111	3·7	2·7
(3) 13. xi. 59	83	7·5	4	3	<90	2·7	1·3

Data marked (1) and (2) obtained simultaneously after acclimatization overnight to the temperatures shown. Data marked (3) obtained simultaneously after acclimatization for 3 days.

* Spawning occurred overnight only when there was a significant drop in temperature.

obtained for quantity and activity of sperm shed. However, worms acclimatized for a similar period to 13·5–14·5° C gave correspondingly poor results, in relation to other assays in the same group. While *Arenicola marina* can survive for some time in the laboratory, it appears that their ability to respond to injection of spawning stimulant rapidly deteriorates. Normally the experiments described in this paper were carried out on the day the worms were collected or on the following day.

DISCUSSION

The first experiments showed that the administration of crude tissue extracts to male worms brings about breakdown of the sperm morulae to free spermatozoa in the coelom and subsequent shedding. As noted previously, the

presence of free spermatozoa in quantity in the coelom has otherwise only been observed in worms actually spawning or in worms which have been damaged and are undergoing histolysis. It may be that shedding, both in response to the injection of tissue extracts and under natural conditions, merely results from this breakdown of the sperm morulae to free spermatozoa in the coelom. The ciliated funnels of the nephromixia must reject the gametes during their development in the coelom. Once, however, the spermatozoa are freed from the morulae the ciliary currents may be capable of handling them and passing them through the nephromixia to the exterior. Worms which spawned in response to the injection of less potent or diluted extracts, tended to shed only a small quantity of sperm after a more than usually prolonged period of time. The interpretation of this may be simply that with weaker concentrations of the active substance only a small number of morulae are broken down before the active substance becomes exhausted. As a result, the nephromixia may fill up less rapidly than when numerous free spermatozoa are present in the coelom, and in total the quantity of free sperm shed is relatively small.

The method by which tissue extracts bring about the disintegration of the morulae has not been investigated. As the active substance is in the fatty acid fraction it might be argued that it is a pH effect. This is considered unlikely as the amount of free acid in crude extracts or in the whole fat is probably small. Furthermore, experiments in progress indicate a greater chemical specificity of the spawning stimulant in that saturated fatty acids appear not to induce shedding. The possibility that the breakdown of the morulae may be brought about indirectly cannot be overlooked. While the release of the spermatozoa in the coelom may be the main factor in bringing about the shedding of male worms the role of the nephromixia is probably less passive than has so far been suggested. This is indicated by the fact that sperm morulae may be present in the first sperm spawned, particularly when less potent extracts are injected.

The mode of action of tissue extracts in bringing about shedding in the lugworm appears to be quite different from that reported for echinoderms. Palmer (1937) has shown that the injection of tissue extracts into the perivisceral cavity of ripe specimens of *Arbacia punctulata* produced contraction of the smooth muscle of the gonad and shedding of the gametes. A muscle stimulant is also present in crude extracts of lugworm tissues but this appears to have no significance in the stimulation of spawning.

Much information is available in the literature with regard to the relationship between environmental conditions and the onset of spawning in marine invertebrates (see, for instance, Orton, 1920). Physiologically, however, changes in the environment can act only as a trigger for the spawning mechanism. There must in addition be some 'pathway' within the organism (neural or humoral) by which shedding of the gametes is immediately effected.

While the spawning stimulant which can be extracted from the tissues of the lugworm will provide a useful tool for the examination of the spawning mechanism, its significance largely depends on whether it can be regarded as providing the normal internal pathway by which spawning is induced. The work of Galtsoff (1940) on oysters indicates the dangers in coming to hasty conclusions with regard to the specificity of stimuli which may induce shedding in males of invertebrate species. However, the spawning stimulant described in the present work was extracted from the tissues of the worm and it appears to be a specific fraction of tissue extracts which is effective. This, together with the normality of the spawning reaction induced, tends to support the hypothesis that the spawning stimulant in tissue extracts does provide the internal stimulus in the spawning of male lugworm. Further support may be gained from the similarity between temperature conditions prevailing on the shore during the breeding season and those influencing the effectiveness of extracts in inducing spawning in the laboratory. The breeding season on the shore coincides with *minimum* air temperatures of the order of 32–46° F (0–8° C) and appears to cease if the temperature stays above 51° F (10·5° C, Howie, 1959). Tissue extracts are most effective in inducing spawning in the range 8 ± 3° C, while variable results are obtained at 12° C (53·5° F) and there is inhibition of spawning at 13° C and above.

It has been shown that the active substance in tissue extracts is present equally in male, female and immature worms and that it is present in the tissues at all times of year. Furthermore, it is to be found in all the major regions of the worm including the coelomic contents. The active substance is therefore a normal constituent of the tissues. However, the fat derived from the gut (even at a lower concentration) and from the coelomic contents proved more potent than the body-wall extract in inducing spawning. Thus, ignoring the coelomic contents, the gut would appear to provide the main source of the active substance. It is only possible to speculate as to how the active substance may be released so that it affects the gametes in the body cavity. This could occur by histolysis, particularly of the gut, similar to that which occurs in *Nereis diversicolor* immediately prior to the breeding season (Dales, 1950). As has already been noted, worms undergoing histolysis, usually resulting from damage inflicted while they were being collected, often show considerable breakdown of the sperm morulae in the coelom. The report by Newell (1948) of a 40% mortality among the lugworm population at Whitstable following upon the breeding season might be significant in this respect. A similar situation may exist in *Amphitrite johnstoni*. Dales (1957) discusses transport and storage of fat in this species and shows that the fat content, particularly of the gut, increases in the spring. It reaches its maximum in August when the gametes, it is inferred, are well developed. In November, immediately after shedding, the fat content of the gut shows a marked decrease. Unfortunately, Dales gives no figures for the fat content of the tissues in September and

October. However, up to August, while the gametes are developing in the body cavity, fat replacement appears to keep pace with, or exceed, utilization. Unless this situation is reversed during the last few weeks prior to the breeding season, it would appear that there is a marked release of fat from the tissues of *Amphitrite* when spawning occurs. The histological condition of *Arenicola* prior to and during the breeding season is now under investigation.

SUMMARY

Macerated tissues of whole *Arenicola marina*, when injected into the body cavity of male worms, induce spawning after a prolonged latent period of, generally, 60–90 min. The nature of the spawning reaction is described. Emulsified tissues were submitted to heat, ultra-filtration, extraction by fat solvents and saponification. The technique is described whereby the products of these treatments were assayed for potency in inducing the spawning of male worms. The active substance proved to be a lipid and upon rigorous saponification the fatty-acid fraction was found to induce shedding, but the unsaponifiable fractions failed to do so. The active substance was also found to be a normal tissue constituent of male, female and immature worms, but it is concentrated in the gut and coelomic contents. Tissue extracts appear to induce spawning primarily by the breakdown of the sperm morulae in the coelom to free spermatozoa, but there may also be some modification of the action of the nephromixia. It is suggested that the active substance found in the tissues provides the 'internal stimulus' by which spawning is normally induced. Evidence for this suggestion is that it is derived from the tissues of the worm and that it appears to be a specific chemical fraction of the tissues which is effective. In addition, tissue extracts induce a normal spawning reaction and there appears to be a relationship between the temperatures at which the active substance is most potent in inducing shedding in the laboratory and those prevailing on the shore during the breeding season. The possible mode of release of the active substance is discussed.

ADDENDUM

In experiments carried out since this paper was written, it has been found that in estimating the density of sperm suspensions using an 'EEL' colorimeter a stricter relationship between sperm densities (as estimated by haemocytometer counts) and colorimeter readings is obtained if a yellow filter (*ca.* 575 mμ) is used.

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APPENDIX A

Preparation of ether extracts

Mixed samples of males, females and worms containing no genital products were macerated in an MSE 'Ato-mix 800' blender. The efficiency of the maceration was much increased by the sand blast produced by the gut contents. The resulting emulsion was divided into 50 ml. portions and each was continuously shaken with 100 ml. ether for about 1 h. The ethereal solution was separated by centrifugation. The residue was re-extracted with further portions of ether until the ether was colourless. The combined ethereal extracts were then filtered through Whatman no. 1 filter-paper or through Carlson 'EK' asbestos sterilizing filter film. The solute was recovered by distillation of the ether. Final drying to constant weight was carried out in an oven at about 40° C for more than 3 days.

Preparation of benzene extracts

Samples of worms were macerated as described above. The emulsion was then dried in an oven at 80° C and the residue, which contained a quantity of sand, was ground down in a mortar. This was then extracted by benzene in a soxhlet apparatus until the solvent was almost colourless. Alternatively, the emulsion was dehydrated by co-distillation with benzene (Bergmann, 1949). When no further water came over the benzene was decanted from the residue. The residue was then re-extracted with benzene in a soxhlet apparatus until the solvent was almost colourless. The benzene fractions from the co-distillation and soxhlet extraction were combined. In both methods the solvent was filtered and the solute recovered as described for ether extracts.

Preparation of acetone extracts

The emulsion after maceration (50 ml.) was extracted with 100 ml. boiling acetone. The solution was then decanted off and evaporated to dryness. The residue was re-extracted in 100 ml. hot acetone. The solvent was subsequently paper or ultra-filtered while hot and the residue recovered by evaporation.

Saponification of the lipid

The lipid, after benzene extraction, was saponified by the method described by Bergmann (1949). The fat (10·8 g), potassium hydroxide (11 g), methanol (80 ml.) and water (30 ml.) were refluxed for 6 h. The solution was then diluted with water (250 ml.) and extracted with ether (4 × 50 ml.) until the ether was almost colourless. The ethereal solutions were washed with water, dried over sodium sulphate and the ether removed by distillation. The residue was recrystallized from ethanol to give the 'cholesterol' fraction. The mother liquors, which probably still contained some sterol, were evaporated to give the 'mother liquor' fraction. The original solution after ether extraction was acidified and extracted with ether (4 × 50 ml.). The ethereal solution was again washed with water and dried over sodium sulphate. The ether was removed by distillation. The residue was called the 'fatty acid' fraction. The author is indebted to Dr T. B. H. McMurry for carrying out this fractionation.

The fats recovered by these various methods were made up for injection in arachis oil or 0·5 M saline in the same concentration as that in which they occurred in the original tissue emulsion. It was found that dispersion of the fat in both carriers was much aided by warming the fat and the carrier before mixing them.

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FURTHER OBSERVATIONS ON THE FINE STRUCTURE OF *CHRYSOCHROMULINA ERICINA* PARKE & MANTON

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(With 29 Figures on Plates I–VII)

The observations to be presented supplement the previous taxonomic description of the species (Parke, Manton & Clarke, 1956) in several respects. *Chrysochromulina ericina* is one of six marine species attributed to *Chrysochromulina* by Parke, Manton & Clarke in 1955 and 1956, and described with the aid of low resolution electron microscopy of external morphology only. Subsequent observations on two further species of *Chrysochromulina* (Parke, Manton & Clarke, 1958, 1959), which included some information on microanatomy from higher resolution electron microscopy of thin sections, had revealed structural differences between them which could not be taxonomically assessed without a broader basis of comparison. Since the easiest way of supplying this is to re-investigate with newer methods some of the existing named species, *C. ericina* was selected for this purpose as an obvious first choice.

The structural differences found in the two anatomically investigated species (*C. chiton* and *C. strobilus*) include the number of fibres in the core of the haptonema (7 in *C. chiton* and 6 in *C. strobilus*), the form and position of the pyrenoid (immersed in *C. strobilus*, but an external diverticulum on the chromatophore in *C. chiton*) and the very peculiar character of the Golgi structure in *C. strobilus*, of a kind which is either undemonstrated or absent in *C. chiton*. The new anatomical observations have been directed to ascertaining the facts on these particular matters for the species under investigation, but in addition *C. ericina* possesses a type of superficial scale (long tapering spines with flared base) which has not been encountered so far in other species but which cannot be completely described without the aid of sections. The difficulty of interpreting the morphology of the scales without this information is therefore the special reason for selecting this species for further study.

[1] Development Commission Fellow seconded by the Marine Biological Association to Leeds University for the academic year 1959–60 to work on this and other problems.

MATERIAL AND METHODS

The material is exactly the same as before, consisting of cultures representing the holotype of *C. ericina* supplied from Plymouth as required. The electron microscopical methods are exactly as in Parke *et al.* (1958, 1959), but advantage has been taken of an opportunity to apply additional light optical methods and only these need be explained in detail.

In 1956 photographic observations with the light microscope had been limited to dried cells, in which the spines could be seen, though with difficulty, and to a few examples of cells fixed in iodine and photographed under oil immersion to demonstrate ingestion of carbon pellets supplied to the culture in the form of Aquadag for the purpose of investigating phagotrophic feeding.

By the use of dark ground illumination it has now been possible to photograph various conditions of the haptonema in iodine-fixed material and by the use of anoptral contrast to photograph the organism alive.

The photographs of living cells reproduced (Pls. I and II) have been made with a Reichert Zetopan microscope fitted with a 1·8 mm anoptral contrast, achromatic, oil-immersion objective (× 100) N.A. 1·25, used with an aplanatic × 10 eyepiece and a Polyphos condenser. With a 30 W light source and no filters the possible exposure times on Ilford H.P.S. plates are $\frac{1}{5}$ and $\frac{1}{2}$ sec. This is sufficiently short for all but rapidly swimming cells. Slight flattening of living cells by coverslip pressure, easily obtained by gradual evaporation of water from an unsealed mount, facilitates examination of cell contents and it is often instructive to allow cells to flatten until they burst.

NEW OBSERVATIONS WITH THE LIGHT MICROSCOPE

One of the first new observations which could easily be made with anoptral contrast was to trace the differences in incidence of spined scales from cell to cell. Substantial differences occur. Figs. 6 and 7 are two successive conditions of a cell which is being squeezed to death by pressure from the coverslip, and it is at once obvious that the scales are conspicuous objects which can be counted exactly under this treatment. The numbers actually encountered varied from 0 to 24. In cultures sent by rail from Plymouth a majority of cells without spines is always present, doubtless in part as a direct consequence of the disturbances involved in the journey. On subculturing the average number of spines per cell builds up rapidly for the first couple of weeks, after which it falls again until in old cultures (1–3 months) cells with many spines may be difficult to find though they can always be recovered in small numbers from the coolest part of the culture near the bottom of the flask. Some actual figures obtained from counts on two different ages of culture are recorded in Table 1.

For study of the internal organs in intact living cells, the absence of scales is an advantage and the cells shown in Figs. 1–4 are in this condition. The bifid chromatophores are conspicuous, see especially Figs. 1 and 3. When a cell has actually burst by flattening (Fig. 4) the chromatophores swell and become circular in face view before eventually disintegrating. For a short time before this happens striations radiating from an unstriated central area are conspicuous (Fig. 4); the latter almost certainly corresponds to the pyrenoid which in other views, notably Fig. 5, may appear dense (i.e. bright).

TABLE 1. COMPARISON OF SPINE NUMBERS IN AN OLD CULTURE ($2\frac{1}{2}$ MONTHS) AND A YOUNG CULTURE (2 WEEKS)

	No. of spines per cell												
	0	1	2	3	4	5	6	7	8	9	10	11	12
No. of cells													
Old culture	65	19	10	6	1	2	5	.	2	1	.	.	.
Young culture	.	2	3	5	8	4	5	4	4	2	3	2	2

	No. of spines per cell												
	13	14	15	16	17	18	19	20	21	22	23	24	25
No. of cells													
Old culture	.	.	.	.	.	.	.	1	.	.	.	.	.
Young culture	.	1	2	.	1	2	1	2	.	1	.	2	.

The nucleus is always inconspicuous and is very frequently masked by one lobe of a chromatophore; it is, nevertheless, distinct in Fig. 2 (*N*) and faintly visible in Fig. 3 (*N*), in both cases the spherical nucleolus and the curve of the nuclear membrane being the only ascertainable features.

The cell membrane is best seen in Fig. 5 in which, under pressure, the underlying cytoplasm has separated from it in several places. Two undischarged muciferous bodies have maintained contact with the inner side of the cell membrane on the left, where they can be seen as small bright spheroids. Examples of discharged muciferous bodies are included in Fig. 10 (arrows) where they take the form of delicate strands sometimes ending in a spheroid and often of very considerable length.

The only other internal organs which can be identified with certainty are some spherical oil droplets (very bright in all these cells) and a large sphere of alcohol-soluble material which we believe to be leucosin (*L* in Figs. 2–4) sometimes with a central inclusion of unknown nature. Other vesicles of various sizes and with or without contents can be seen from time to time (e.g. Figs. 3 and 4) though without certainty as to their nature. Additional cell organelles known to be present on other evidence, notably mitochondria and Golgi bodies, are not demonstrable by these methods and there is therefore nothing to add to the previous account (Parke *et al.* 1956, p. 395).

The various manifestations of cell movement recorded from unflattened living cells assembled in Plate II compare closely with drawings previously

published and need little further explanation. Attention should, however, be directed to the pseudopodia-like bodies on the cell surface in Fig. 2. They may perhaps be the same as protuberances described as flowing out in relation to phagotrophic feeding (p. 397 of the previous paper), though as seen here they are relatively static structures on a small proportion only of the motile cells. They have not been seen in any special relation to ingestion or egestion and with transmitted light they are so transparent as to be virtually invisible. They are therefore perhaps a previously undescribed type of temporary surface feature.

Explanation of Plates I and II

Chrysochromulina ericina Parke & Manton, living cells with anoptral contrast (× 2000)

I

Fig. 1. A spineless cell with two stationary flagella of equal length and no haptonema; two bifid chromatophores near the flagellar pole (a characteristic position) with a third laterally.

Fig. 2. Amoeboid swimming cell with one beating flagellum; the second flagellum has been withdrawn into the cell and the haptonema has been shed; two chromatophores, the nucleus (*N*), a leucosin vesicle (*L*) and three bright fat droplets are visible.

Fig. 3. A cell showing the bifid shape of the chromatophores more clearly and with the nucleus (*N*) faintly discernible against the lower lobe of the right-hand chromatophore in the centre of the cell; other identifiable cell inclusions are two bright oil droplets of different size and a leucosin vesicle (*L*); a few spined scales visible in the background (left).

Fig. 4. Remains of a cell immediately after bursting; the leucosin vesicle (*L*) and fat droplets still intact, the four chromatophores undergoing post-mortem changes which include the appearance of peripheral striations round a central clear area marking the position of the pyrenoid.

Fig. 5. Cell under slight pressure showing the body membrane raised up from the cytoplasm; two undischarged muciferous bodies are still attached to the inner side of the body membrane (left); chromatophores near the flagellar pole viewed from the side show the pyrenoids (*Py*) as bright inclusions; fat droplets and other cell inclusions visible; detached scales in the background.

Figs. 6, 7. Two successive stages of a cell under pressure until it bursts (Fig. 7). In both, a considerable array of scales detached from the surface are visible in the field.

II

Swimming cells (× 2000)

Figs. 8, 9. Two cells with extended haptonema (*H*) and beating flagella; in Fig. 8 the flagellar beat is relatively slow and in Fig. 9 faster.

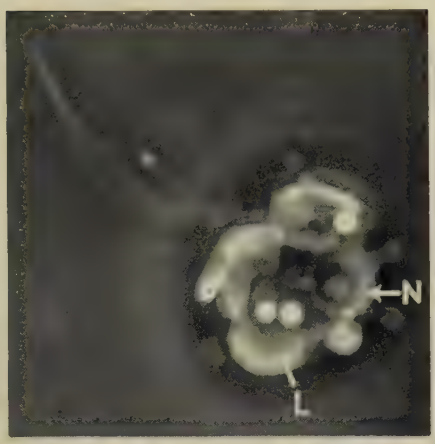
Fig. 10. Cell with the two flagella beating slightly and the haptonema (*H*) partly uncoiled; three thread-like discharges from muciferous bodies are present in the field (arrows).

Fig. 11. Stationary cell, with haptonema (*H*) extended at the base but tightly coiled at the tip.

Fig. 12. Stationary cell with flagella beating slightly and the haptonema (*H*) tightly coiled.

Fig. 13. Cell fixed with iodine in KI and photographed with dark-ground illumination to show the three appendages in the fixed condition (for other views see Figs. 17–20).

1



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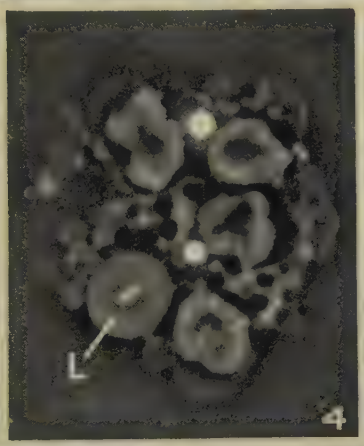

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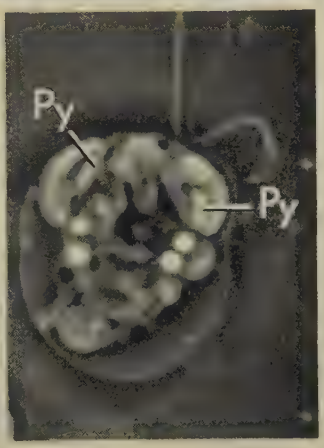

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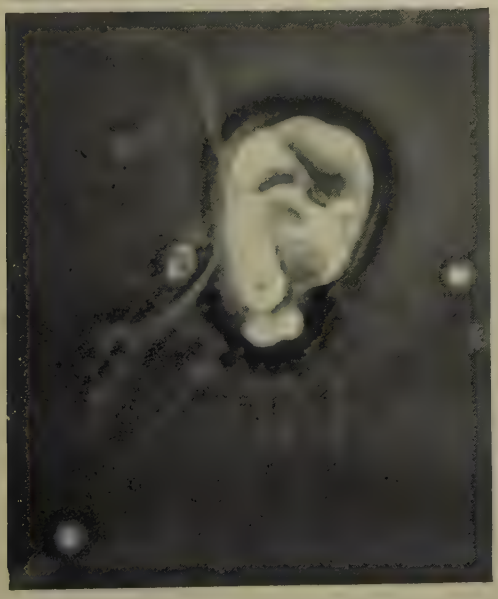

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7

(*Facing p.* 148)

8

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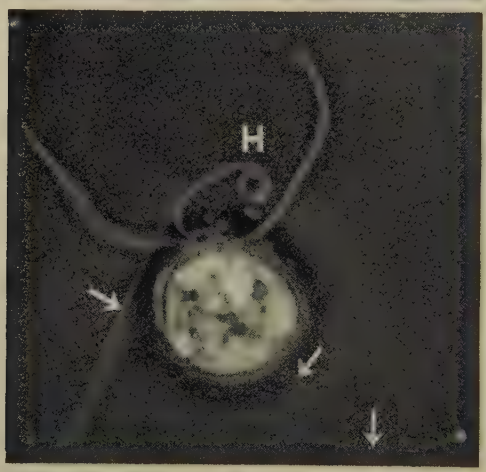

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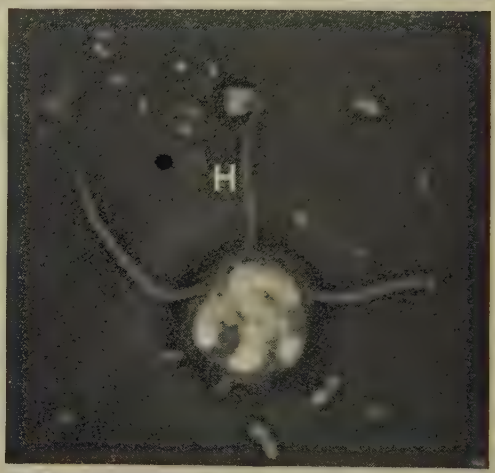

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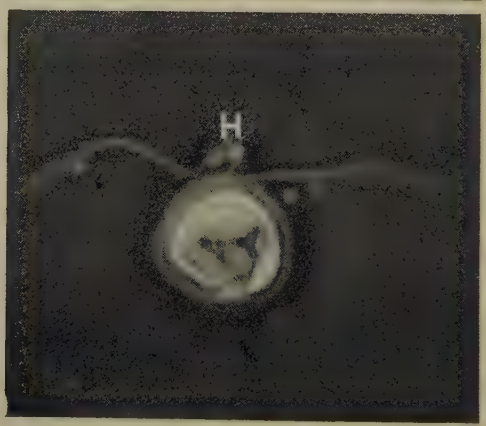

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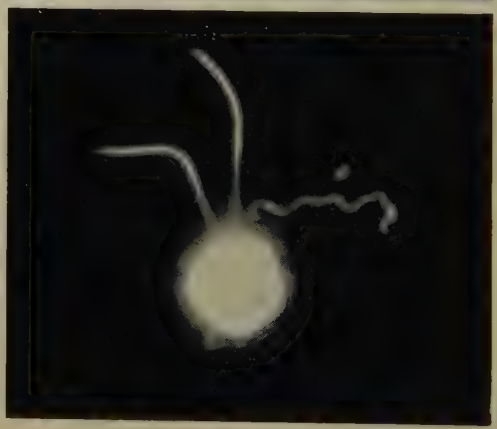

13

NEW OBSERVATIONS WITH THE ELECTRON MICROSCOPE

The haptonema

This is commonly detached from the cells in this species after osmic fixation and therefore the best views of it in position are those taken in life (Plate II) or with the light microscope after iodine fixation (Figs. 17–20). It is, nevertheless, not difficult to pick up intact haptonemata lying loose on the field in shadow-cast preparations and one example is reproduced in Fig. 14. This is doubtless broken off from the cell at or near the point of attachment but the coiled condition involving 8–9 gyres of a solenoid is well preserved.

A similar, detached, haptonema is shown in section in Figs. 15 and 16. Fixation is far from perfect since both blistering and splitting of membranes has occurred locally on a considerable scale. Nevertheless, the best oriented profiles (arrows in Fig. 16) show three concentric membranes surrounding a ring of seven fibres. This is exactly the structure previously described for *C. chiton*, though not exactly as in *C. strobilus*.

Pyrenoids

Figs. 26, 28 and 29 show with sufficient clarity that the pyrenoid here is sunk in the centre of the chromatophore as in *C. strobilus*. The pyrenoid itself consists of an opaque mass traversed by canaliculi and forming a central swelling in the region which, in a burst cell (Fig. 4), appears featureless. The plastid lamellations proper are probably not individually visible with the light microscope even when striations appear as in Fig. 4, but they can be seen in Figs. 26, 28 and 29 in which they resemble those of other members of the Chrysophyceae.

The unambiguous evidence on pyrenoids obtainable with the electron microscope is in marked contrast to the situation with visual light in which pyrenoids are exceedingly difficult to detect with certainty without the aid of anoptral contrast (see especially Fig. 5). It now seems probable that several references to 'pyrenoid-like bodies' in the literature and in the earlier descriptions of these species (e.g. Text-fig. 1 and p. 394 of the previous paper) must in fact represent some other, as yet unidentified, cell component.

Golgi structure

This can be seen in profile in the centre of Fig. 26, occupying a substantial part of the cytoplasm between the two chromatophores. The peculiarity of structure previously encountered for the first time in *C. strobilus* is again displayed. There is nothing yet to add to the structural interpretation given under that species (Parke *et al.* 1959), and it is sufficient here to have demonstrated similarity.

The nucleus

It is characteristic of this species to have the nucleus in a large proportion of cases apposed to the inner surface of one lobe of a chromatophore, hence its invisibility with the light microscope in a majority of cells. This position is well seen in Fig. 28 and still better in Fig. 29. When most closely apposed to the chromatophore there seems to be a local confluence of membranes resulting in disappearance of the cytoplasmic boundary of the perinuclear space between the two organs. A common cytoplasmic boundary covering both nucleus and chromatophore can then be detected at the two ends of the nucleus (Fig. 29). This feature, which is by no means confined to the present species, is of some electron microscopical interest and will be more fully demonstrated elsewhere.

Other cell inclusions

The only other cell inclusions to which attention need be directed are (*a*) certain fibrous connexions from the bases of the appendages, and (*b*) muciferous bodies. The former are represented in Figs. 27 and 28. A fibrous connexion passing from the lower end of a flagellar base diagonally out to the cell surface is seen in Fig. 27 (arrows), while in Fig. 28 two fibrous connexions, one on each side of the remains of the haptonema base, pass

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Explanation of Plates III–VII

Chrysochromulina ericina Parke & Manton

III

Structure of the haptonema

Fig. 14. A detached coiled haptonema from a shadow-cast whole mount of cells killed directly on a prepared carbon film with osmic vapour. Electron micrograph H 7037, magnification × 12,000.

Fig. 15. Section of a detached coiled haptonema. Electron micrograph H 6860, magnification × 20,000.

Fig. 16. More highly magnified part of the preceding showing the three concentric membranes and seven central fibres in the most favourable profiles (arrows). Electron micrograph H 6863, magnification × 50,000.

Figs. 17–20. Intact cells killed with iodine in KI and photographed with the light microscope under dark ground illumination to show various stages of coiling of the haptonema, magnification × 1000.

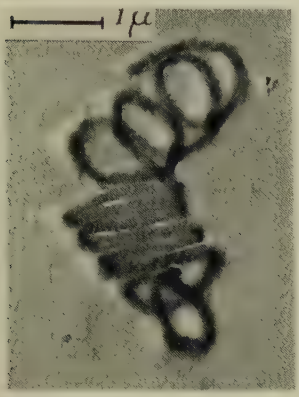
IV

Scale structure

Fig. 21. Lower end of a spine to show the details of the flared base in a shadow-cast whole mount. Micrograph H 7035, reversed print, × 30,000.

Fig. 22. Median longitudinal section through a spine (selected from a series) with parts of other spines in the background (right) and the edge of a cell (left). Micrograph H 7022 magnification × 30,000.

Fig. 23. Group of plate-scales near the base of a spine to show the pattern of radiating ridges characteristic of one face (contrast with fig. 25). Micrograph H 7045, reversed print, magnification × 25,000.



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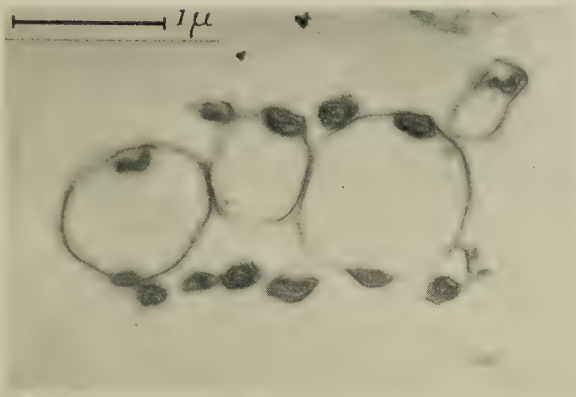

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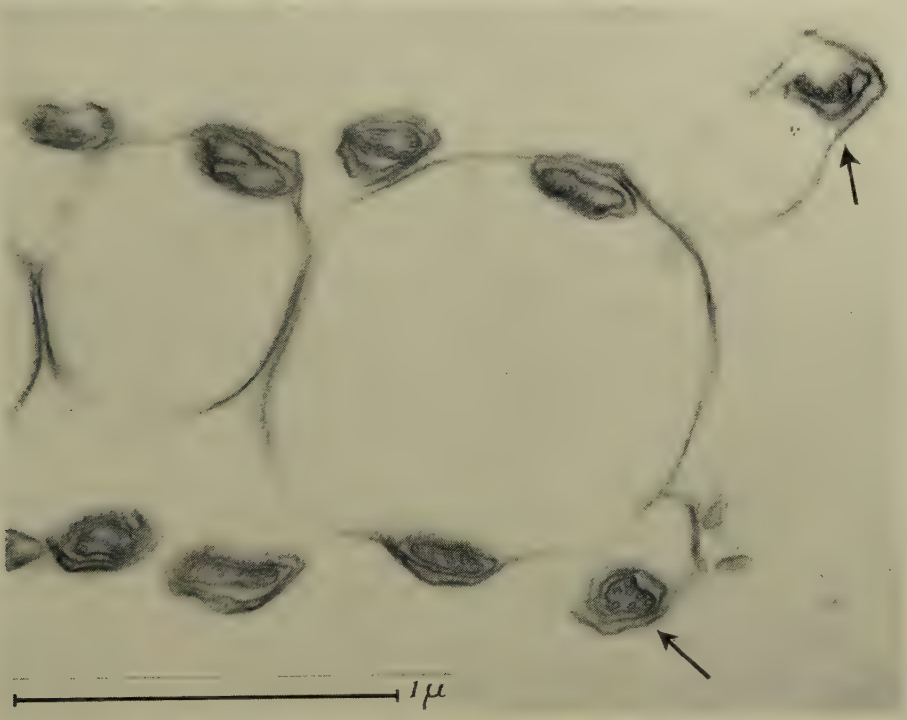

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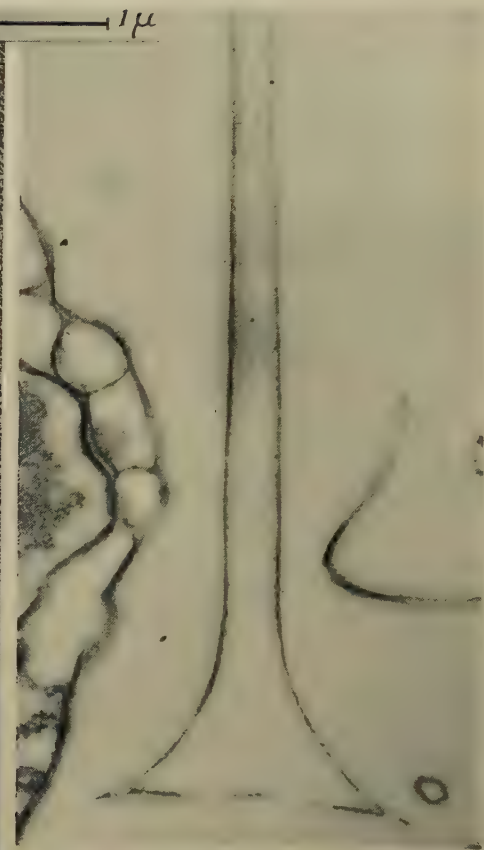

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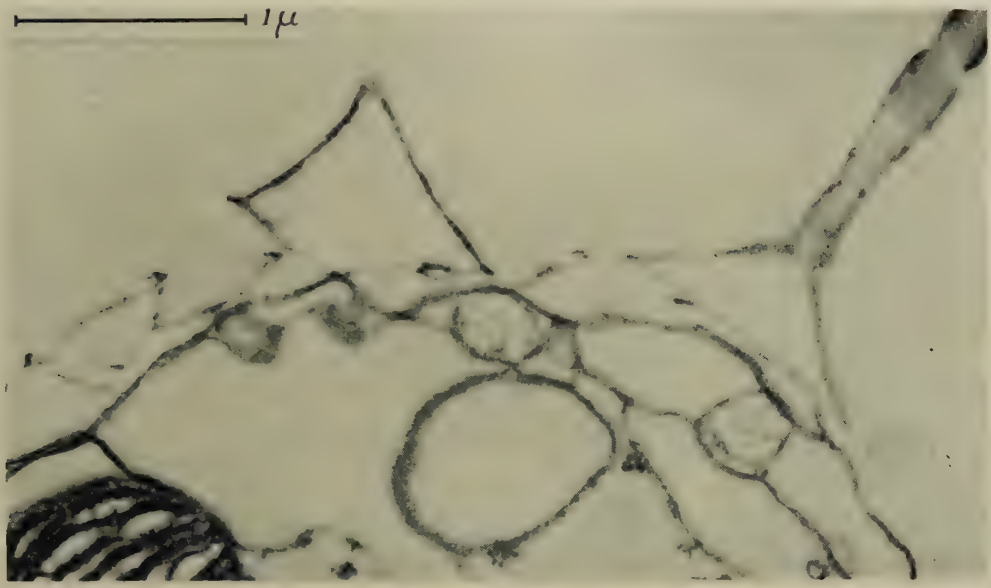

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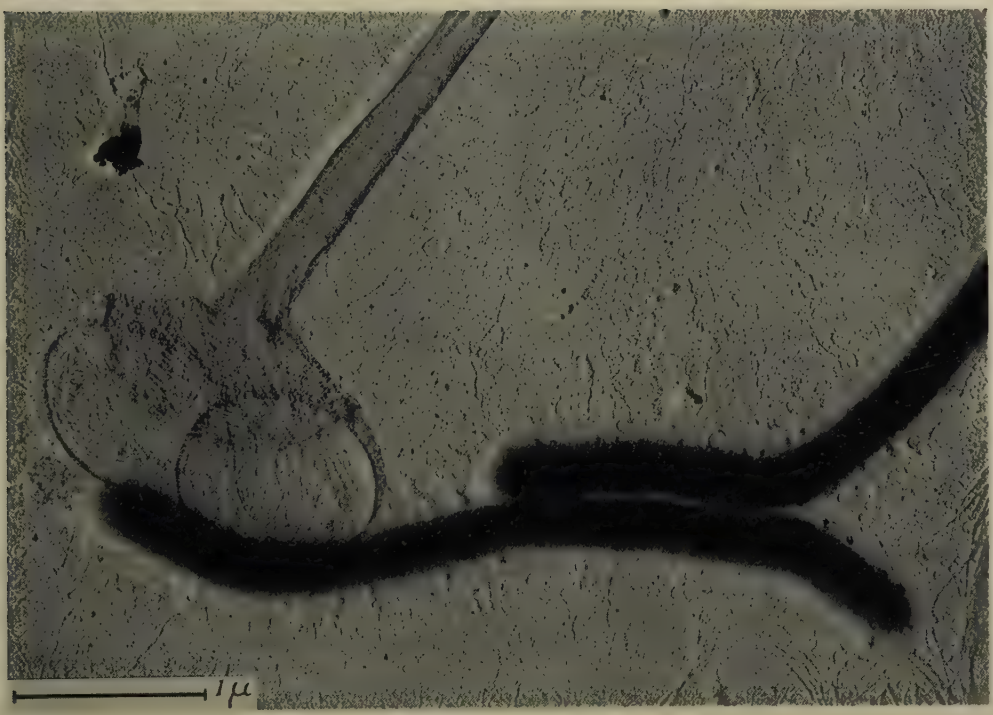

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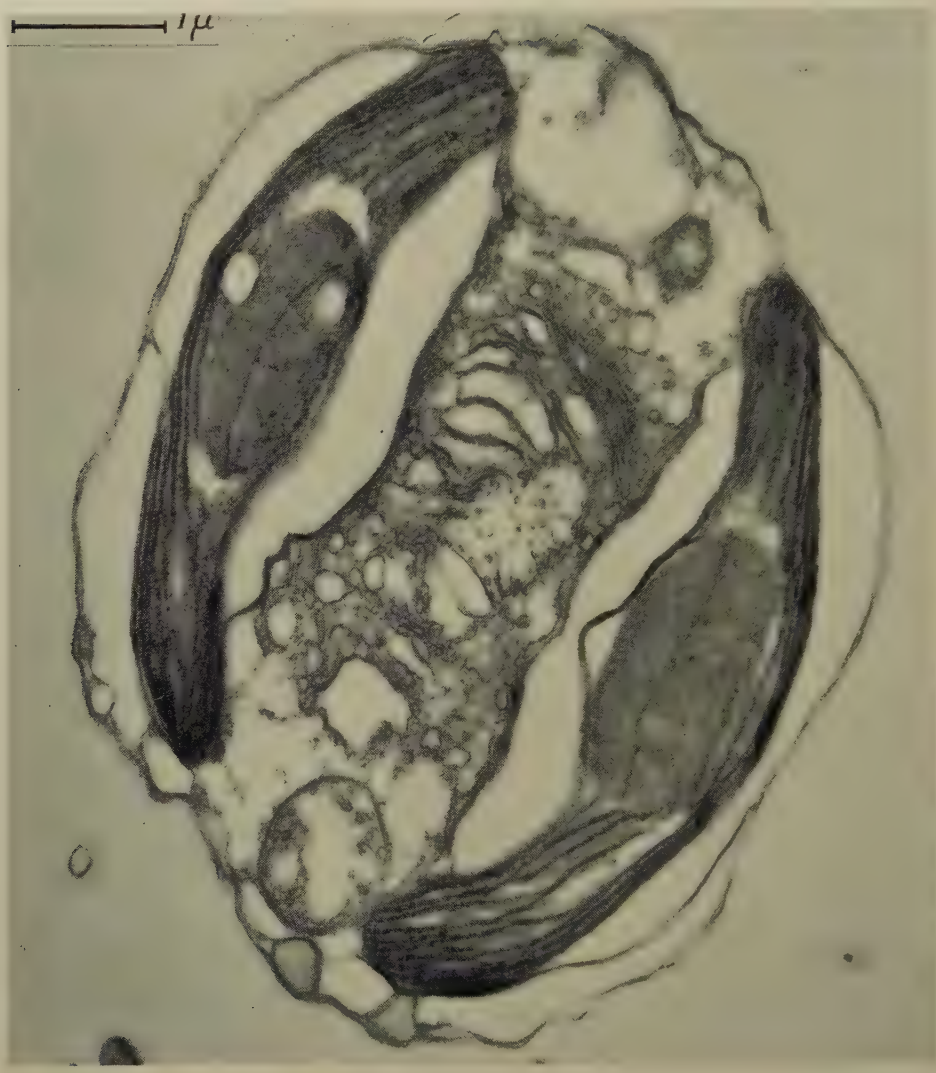

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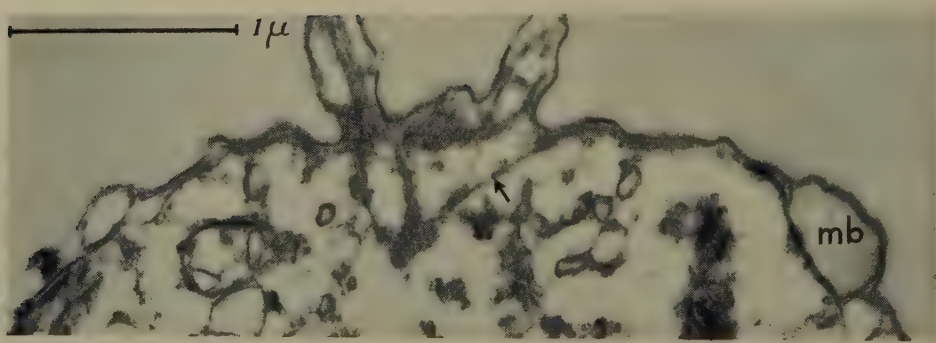

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28

29

outwards under the body membrane to end on the surface of the adjacent chromatophores.

Undischarged muciferous bodies are visible on the cell surface below the body membrane in several sections, notably Fig. 26 (lower end) and Fig. 27 (*mb*). These may usefully be compared with the photograph of the live condition in Fig. 5. They consist of membrane-bounded spaces filled with semi-opaque contents but without other discernible structural features. After discharge, the contents of muciferous bodies appear as substantial cylinders of opaque material of uniform diameter when examined in whole mounts with the electron microscope (Fig. 25), in striking contrast to their very delicate appearance in life (Fig. 10). Their functional significance is unknown.

Explanation of Plates III–VII continued

Chrysochromulina ericina Parke & Manton

V

Fig. 24. Part of a cell with scales still in position on the surface; these include the bases of two spines (neither in median section) and an array of plate-scales with the inflexed rim projecting outwards relative to the cell surface. Cell contents include the edge of a chromatophore (left), some apparently discharged muciferous bodies (surface) and a few large membrane-bounded spaces. Micrograph H 8709, magnification × 30,000.

Fig. 25. The contents of two discharged muciferous bodies (dark) and a small group of plate scales near the base of a spine to show the raised rim and surface pattern characteristic of the opposite face to that displayed in Fig. 23. From the evidence of sections (Fig. 24) the face displayed in the plate-scales of Fig. 25 is the outer one. Micrograph H 7050, × 25,000.

VI

Cell anatomy

Fig. 26. Section passing through the Golgi area (centre) and with lamellations and a central pyrenoid visible in the two lateral chromatophores; a mitochondrion and two undischarged muciferous bodies present at the bottom end of the section. Micrograph H 6871, magnification × 20,000.

Fig. 27. Part of the flagellar pole of a cell to show a fibrous strand (arrow) passing from the base of a flagellum to the cell surface; an undischarged muciferous body (*mb*) present beneath the cell membrane near the edge of the field on the right. Micrograph H 8815, magnification × 30,000.

VII

Fig. 28. Section showing the flagellar pole in oblique longitudinal view (left), two chromatophores and the nucleus (*N*), with traces of Golgi vesicles just grazed by the section in the central cytoplasm. The flagellar bases are not included but remains of the haptonema base are present from which two lateral fibres (arrows) pass outwards below the body membrane to end on the surface of the adjacent chromatophores. Micrograph H 8680, magnification × 15,000.

Fig. 29. Transverse section (approximately) of a cell passing through four lobes of two bifid chromatophores against one of which the nucleus (*N*) is apposed; note the shared cytoplasmic boundary between nucleus and chromatophore visible at the two ends of the nuclear profile; in the central cytoplasm a group of mitochondria (left) and an approximately polar view of the Golgi body (right). Micrograph H 6848, magnification × 15,000.

The scales

These are so important in the present context that two Plates (Pls. IV and V) are devoted to them. It is known from the previous account (Parke *et al.* 1956) that both spines and plate-scales are present, the latter greatly outnumbering the former though only the spines can be seen individually with the light microscope. The plate-scales have an easily distinguishable difference of pattern on the two faces (Figs. 23, 25), one side being embellished with radiating ridges extending completely to the edge (Fig. 23), the other side having a pattern of concentric or interwoven lines bounded peripherally by an upturned rim (Fig. 25). The flared bases of the spine-scales resemble the latter surface in their marking but it cannot be known from whole mounts whether the spine itself is solid or hollow and whether the flared base is open or closed at the lower end.

These questions are at once answered by a median longitudinal section of a spine (Fig. 22) from which it is quite clear that the spine is hollow and that the lower end of the flared base is closed by a flat plate. With this information available it is possible to extend the description by utilizing a detail often visible in whole mounts but previously uninterpretable. As may be seen on the bottom edge of the base of the spine in Fig. 21 and again on the left-hand edge of the base of the spine in Fig. 23 there is sometimes a marking resembling a milled rim present on part of the curved lower margin of the flattened spine. It is now obvious that this cannot be a milled rim since it is not uniformly displayed. What we are almost certainly seeing is part of the outer edge of the plate closing the base of the spine which must be carrying a pattern of radiating ridges on its surface of the same type as on the lower face of the plate-scales (Fig. 23).

That this description correctly interprets the orientation of the plate-scales with respect to the subtending cell surface is clearly demonstrated by the occasional fortunate section in which a cell is encountered with its scales in position. Such cells are rare because it is probably only at certain times, in relation to the periodic production of new scales, that these are still firmly enough attached to resist the mechanical disturbance of centrifugation. One example is reproduced in Fig. 24, and it includes both spines and plate-scales. In all the latter the slightly inflexed rim is directed outwards relative to the subtending cell surface. This demonstrates that the outer face is indeed that represented by Fig. 25 and the face against the subtending cell that of Fig. 23. This arrangement, it may be said in passing, is exactly the same as that deduced on other evidence from a different type of spined scale in *C. alifera* and *C. ephippium* described at the same time as our present species in 1956 (Parke *et al.* 1956).

DISCUSSION

The new observations on scales are probably the most important single contribution to botanical knowledge which *C. ericina* has provided. A two-layered scale structure had been inferred from morphological evidence for spines of a very different type in *C. ephippium* and *C. alifera* (Parke *et al.* 1956), and two separate layers had been directly seen in sections of the very large saucer-shaped scales of *C. chiton* (Parke *et al.* 1958). This concept could not, however, be further extended until the information regarding the very special type of spine present in *C. ericina* could be completed. The difference between plate-scales and spines in this species represents the most extreme example of scale dimorphism yet encountered in *Chrysochromulina* and it could not be known *a priori* whether the spines here might represent an entirely new category of surface appendage. This is, however, undoubtedly not the case. It is now clear that the spines and plate-scales in this species are essentially similar, both being fundamentally two-layered plates which, in the case of the spines, have become modified by a spatial separation of the two layers except at their margin and by an enormous hypertrophy of the outer layer which is not shared by the inner layer. Since changes of relative dimensions in parts of an organ are always some of the easiest modifications to bring about there is no difficulty in regarding the morphological differences between the scales of all four species for which the facts have been directly ascertained (*C. ericina*, *C. alifera*, *C. ephippium* and *C. chiton*) as relatively minor variants on a common ground plan. Since this ground plan itself is a simple one from which chemical modification by mineralization seems to be entirely lacking it seems likely to be relatively primitive in a group (Chrysophyta) in which extreme complexity of scale structure is often found (the Coccolithophorids and *Synura* are well known examples). This conclusion will undoubtedly be relevant to an eventual appraisal of the phyletic position of haptonema-bearing organisms within the class.

It had been hoped that the very large size of the spines in *C. ericina* would make it possible to ascertain some facts about their mode of production by examination of sections. This hope has however not been realized since the absence of mineralization makes recognition of scale fragments encountered in unusual places virtually impossible. It is nevertheless perhaps significant that very large membrane-bounded cavities are sometimes encountered in cells (cf. Fig. 24) and that the arrays of small or middle-sized vesicles present in some cells of *C. strobilus* and *C. chiton* respectively are never found. This suggests that there is a general correspondence between scale size and vesicle size in all three species, which is a fact consistent with, though of course it does not prove, a derivation of scales from internal vesicles and not directly from the cell membrane. Such a production of scales from internal vesicles has recently been directly demonstrated in the unrelated case of *Micromonas* (Chlorophyceae) (Manton & Parke, 1960).

With regard to the other anatomical observations, these can be amplified by the verbal statement that sections of both *C. ephippium* and *C. alifera* have been examined in a preliminary way, and both species found to agree exactly with *C. ericina* in the three characters of haptonema structure, pyrenoid structure and Golgi structure to which attention has been specially directed here. Anatomical information is thus now available for five species attributed to *Chrysochromulina* namely, *C. chiton*, *C. strobilus*, *C. ericina*, *C. ephippium* and *C. alifera*. All of these agree in the essentials of haptonema structure although *C. strobilus* is exceptional in having only six internal fibres, the other four species having seven. *C. chiton* diverges widely from the others in pyrenoid structure and perhaps also in Golgi structure although re-investigation of this feature is desirable for this particular species. What, if any, taxonomic significance should be attributed to these differences may be expected to become clearer when corresponding facts are available for the type species of the genus. Since this is expected shortly, further discussion may profitably be deferred.

This work has been carried out in close co-operation with Dr M. Parke, who has supplied the living material and also the photographs taken under dark-ground illumination reproduced as Figs. 17–20. We are indebted to the Development Commission for financial assistance and to the laboratory staffs of both Plymouth and Leeds for technical assistance. In this category special mention should be made of Mr K. Oates, senior technician at Leeds, for making the shadow cast preparations of scales represented on Pls. IV and V, Miss Irene Adams of Plymouth for maintaining cultures, and Miss Sheila Wright and Mr John Greenwell of Leeds for help in the photographic printing.

SUMMARY

C. ericina Parke & Manton has been re-investigated to add salient features of micro-anatomy from the electron microscopy of thin sections and also to add photographs of living cells taken with anoptral contrast light microscopy.

The most important new observations concern the scales which are shown to be essentially two-layered plates in which the layers in the very large spined scales have become separated except at their edges, with the outer layer greatly hypertrophied to produce a hollow spine with a flared base closed at the bottom by a flat plate. The patterns of external marking on the two layers are very similar in both plate-scales and spines in this species and the orientation of both with respect to the cell surface has been demonstrated by a section of the scales *in situ*.

A few new observations are added concerning: (*a*) fibrous connexions between the bases of the appendages and the cell surface or adjacent chromatophores; (*b*) muciferous bodies both charged and discharged recorded alive

and electron microscopically; and (*c*) an apposition of the nucleus to the surface of a chromatophore.

The remaining anatomical observations are limited to demonstration of the position and general character of the immersed pyrenoid, of the presence of 'peculiar' Golgi structure and of the details of haptonema structure with seven central fibres. Identical characters are reported for *C. ephippium* and *C. alifera* from sections which are not reproduced, and a comparative summary is given of the anatomical facts now known for five marine species.

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AN UNDERWATER SURVEY ON THE LULWORTH BANKS

By G. R. Forster
The Plymouth Laboratory

(With Text-fig. 1)

There are still very few descriptions of the fauna of submerged rocks around British coasts. An opportunity to examine such a rock fauna with the facilities offered by the marine survey section of the Atomic Energy Authority Establishment at Winfrith Heath was therefore most acceptable.

A few exploratory dives were made in November 1959 around Worbarrow Bay in depths of 5–8 fm; but the low rock reefs encountered were generally surrounded by sand which would be expected to cause scouring or even undergo mass shifts during winter gales. The fauna in this area might therefore be expected to fluctuate considerably from time to time and would not be suitable for a quantitative survey. Fortunately, thanks to information from Mr A. H. Stride of the National Institute of Oceanography, my attention was drawn to the Lulworth Banks lying 2 miles offshore in Weymouth Bay (see Fig. 1) with depths of 8–10 fm. An 'asdic' and core sampling survey had been made by Mr Stride from R.R.S. 'Discovery II' and from his results it was possible to select the roughest rock areas for a second series of dives in August 1960.

The position at which diving was carried out from 15 to 19 August is—Bats Head bearing 009° True—distance 2·2 sea miles. The Decca co-ordinates for the position are Red G5·95: Purple G56·55. The following transits were also noted: (1) two trees just showing in the Arish Mell gap, between the junction of Rings Hill on the right and Bindon Hill on the left; (2) a pair of beacons on the cliffs at Ringstead Bay in line; (3) the Hardy monument in line with a house on the right of a conspicuous white house, presumably South Down dairy.

These marks can be verified by reference to Admiralty Chart No. 2610. The diving position was temporarily fixed with a marker buoy. The depth was 9½ fm at low water (neaps). The sea bed is rocky, apparently limestone with occasional pockets of sand or gravel. On the rock surface there is a very little fine silt deposited wherever shelter is afforded by algal holdfasts or by the various encrusting animals.

General description of the fixed fauna and flora

Small red algae and fine branched Bryozoa covered most of the rock surface. The algae were thickest on any elevated rocks or reefs, while the Bryozoa predominated on any sloping surfaces. A sample of the algae was identified by Dr A. D. Boney. *Calliblepharis ciliata* (Huds.) Kütz. and *Brongniartella byssoides* (Good. et Woodw.) Schm. were particularly abundant; two other species *Chondria dasyphylla* (Woodw.) Ag. and *Nitophyllum* sp. (*bonnemaisonii?*) also occurred. The commonest Bryozoa were *Crisia denticulata* (Lamarck), *Cellaria fistulosa* Hinks. Among the sponges *Polymastia boletiformis* was very common. *Cliona celata* Grant (massive form), *Raspailia hispida* (Montagu) and *Pachymatisma johnstonia* (Bowerbank) were also frequently met with.

Quantitative survey

A quantitative survey was undertaken by the method previously used for *Echinus* (Forster, 1959). A terylene rope 34 m long is stretched out on the sea bed, the diver then swims along with a 2 m measuring rod recording the numbers of certain species which lie in the area covered by the rod. The count is made on each side of the terylene rope and therefore the total area covered is $4 \times 34 = 136$ m^2. The species selected for counting were: *Polymastia boletiformis*—sponge, *Bispira volutacornis* (Montagu)—sabellid worm, and *Lepralia foliacea* (Ellis & Sol.)—bryozoan. The three species were all fairly common and easily recognized. *Lepralia* presented some difficulty as the colonies varied in size from 3 in. diam. to about 18 in.; it was therefore necessary to make some estimate of the size of each colony. All notes were made in pencil on a sheet of 'Cobex' matt-surfaced P.V.C. The position of the different survey lines is shown on Fig. 1 and the results in Table 1. A total of six counts was undertaken. Position 3 has obviously by far the lowest numbers: it was in fact located on a different type of bottom. Instead of the irregular limestone rock the surface was remarkably flat, in the form of a low reef with very little gradient, probably composed of hardened clay. At the edge of this reef was an extensive area of coarse sand. *Flustra foliacea* (L.) was abundant on the reef surface together with large numbers of small scallops, *Chlamys opercularis* (L.); the scallops swam away in front of the diver in a continuous diverging wave. The close proximity of the sand probably accounts for the different fauna. Apart from 3 the other five positions show reasonable agreement in the number of the three species. If the results were averaged one would expect to find eight *Polymastia*, four *Bispira* and two *Lepralia* colonies in 10 m^2 of rock surface.

Compared to similar areas near Plymouth there was a notable absence of *Laminaria hyperborea* (Gunn.) Fosl. which appeared only close inshore, on rocks raised up 3 or 4 ft from the surrounding reef, at 4–5 fm depth.

Echinus esculentus L. was also absent and only one single *Eunicella verrucosa* was observed. *Bispira* was very much more common than at Plymouth, frequently living in holes in the rock provided by boring molluscs. Dr Eve Southward has identified many copepods which she found on the arms of *Bispira* as *Gastrodelphys clausii* Graeffe.

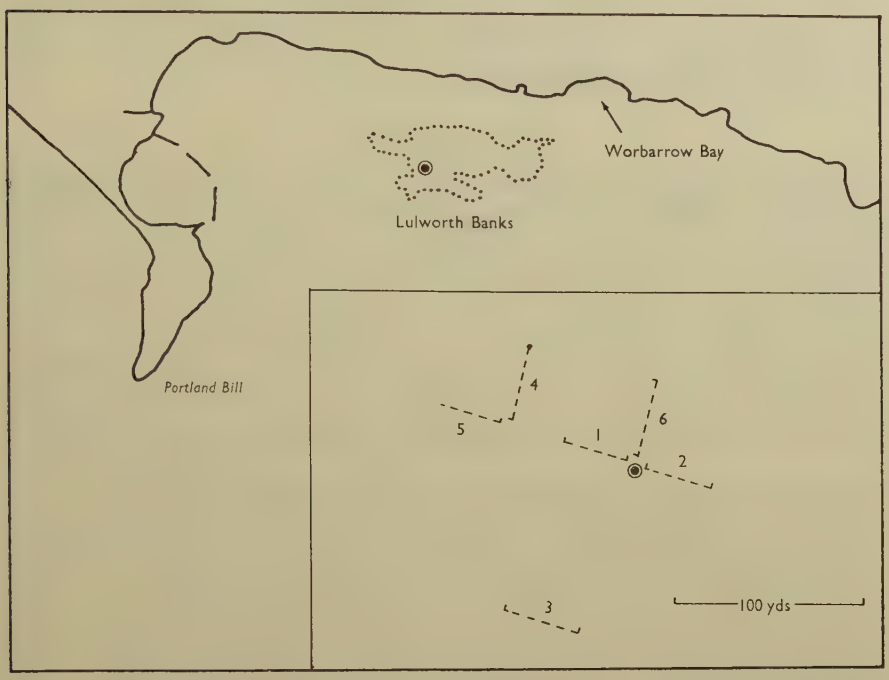

Fig. 1. The outline of Weymouth Bay showing (dotted) the 10 fm contour around Lulworth Banks and the diving positions. The large-scale insert shows the approximate positions of the counts 1–6.

TABLE 1. NUMBERS OF SELECTED SPECIES COUNTED ON THE SEA BED

Survey no.	*Polymastia*	*Bispira*	*Lepralia* colonies 18 in.	12 in.	9 in.	6 in.	3 in.	Total *Lepralia*	Area covered (m^2)
1	173	39	1	1	12	1	6	21	136
2	97	81	1	1	8	2	10	22	136
3	11	8	2	0	1	1	5	9	130
4	105	30	1	0	2	3	5	11	112
5	67	36	1	0	6	9	14	30	136
6	95	86	1	3	5	10	3	22	136
Total (excl. 3)	537	272	5	5	33	25	38	106	656
Mean no. per 10 $m.^2$	8·19	4·15	—	—	—	—	—	1·61	—

I am most indebted to Mr D. J. Scarratt and Mr R. E. Joyce for assistance with diving and with handling the heavy gear; also to the marine survey section of the Winfrith Heath Atomic Energy Research Station for providing a vessel.

SUMMARY

A brief account is given of the sessile animals and algae found by diving on the Lulworth Banks. The numbers of a sponge, a sabellid worm and bryozoan were counted over a total area of 650 m^2.

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SALINITY TOLERANCE AND PERMEABILITY TO WATER OF THE STARFISH *ASTERIAS RUBENS* L.

By JOHN BINYON
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(Text-figs. 1 to 7)

Present-day echinoderms are marine animals and are usually considered to be a stenohaline group, in the sense that they are intolerant of salinities differing greatly from that of normal oceanic sea water. In the Baltic Sea, however, the position is somewhat different. Most groups of echinoderms are to be found in the Kattegat, but the number of species declines eastwards and the asteroids are the only group to extend beyond the Öresund. The farthest penetration is made by *Asterias rubens* which is taken as far east as Rugen Island where the salinity is only 8‰ (Brattström, 1941; Segerstråle, 1949; Schlieper, 1957).

In the British Isles the distribution of echinoderms is fairly well documented. According to Bassindale (1940, 1943), none are to be found in the Bristol Channel above Kilve, where normal salinity conditions obtain. Some post-larval asteroids have, however, been found by Rees (1938) in the Cardiff Roads plankton, where the salinity was 27·1‰. In the Salcombe and Exe estuaries, Allen & Todd (1900) did not report any echinoderms from water of less than 30‰. Percival (1929) and Spooner & Moore (1940) in their Tamar surveys did not record *A. rubens* within the estuary. In north-east England too, echinoderms seem to be absent from the estuaries (Hobson, 1949). Hancock (1955) reported *Asterias* from the River Crouch as far as the western end of Bridgemarsh Island, and the occasional specimen is taken a little higher up the river, where the summer low tide salinity is not reduced. In winter, however, the low tide salinity may fall as low as 18‰, but it is not known whether *Asterias* is also present at these times. Some personal observations made in September 1957, together with the opinion of local fishermen, suggests that in the River Stour *Asterias* is not taken more than 3 miles above Harwich, but full salinity conditions are maintained for a further 8 miles.

The aim of the present study therefore, has been to investigate the salinity tolerance and permeability to water of *A. rubens*. The animals were collected at Whitstable, which is situated at the mouth of the Thames estuary on the north Kent coast. These animals were taken as representative of a population

which in nature does not appear to enter water of significantly reduced salinity, their distribution not extending west of the Pollard Spit area (Newell, 1957, personal communication and personal observations) where the lowest recorded salinity for the year 1956/57 was 28·7‰.

SALINITY TOLERANCE

Some preliminary observations were performed in which five animals were kept in each of a series of tanks containing sea water of different dilutions. The dilutions were made with M/400 sodium bicarbonate and the salinities checked daily by chloride titration and adjusted if necessary. No form of temperature control was available for this series of experiments and the variation encountered was from 14 to 17° C. Food was available to the starfish in the form of *Mytilus edulis*. The water was aerated by means of a small pump fitted with stone diffusers and any dead animals were removed as soon as they were noticed. After 1 week, all those in water of 18‰ had died, whereas those at 25‰ were still in good condition and were feeding.

The lower limit of tolerance was narrowed further by using ten animals in 20 l. of medium for each of a number of different salinities. The LD_{50} after 14 days was taken as the criterion of tolerance. A value of between 22 and 24‰ was obtained. Loosanoff (1945) found that he was unable to affect the lower limit of acclimatization of *Asterias forbesi* (Desor) by the protracted dilution of the medium. Similarly, the value obtained here for *A. rubens* was not affected by diluting the medium so as to cause a fall in salinity of 1‰ per day.

WEIGHT REGULATION AND PERMEABILITY TO WATER

The time course of the weight changes of *A. rubens* when placed in diluted sea water has been followed on only two occasions. In 1934, Bethe placed the animal in 75% sea water and found that there was a maximum gain in weight of 10% in from 3 to 6 h, decreasing to the original value in from 6 to 24 h. Similar results were obtained by Maloeuf (1938) who immersed *Asterias* in 50% sea water. The maximum increase in weight occurred after about 7 h, falling back to normal in from 20 to 50 h. As these animals were shown, by measurements of the internal osmotic pressure, to be isotonic with the medium after they had returned to their original weight, it may be supposed that both water and salts had been lost across the body wall, the animal lacking any morphologically differentiated excretory organ. Maloeuf concluded that *A. rubens* is incapable of any osmoregulation; his graphs, however, and those of Bethe, suggest that it is capable of weight regulation.

Similar experiments to these have been performed on Whitstable animals. In view of the extreme delicacy of the distended starfish, special precautions were taken during the weighing of the animals. These consisted of keeping

them for the experimental period in small plastic cages suspended at the corners in the diluted media. When removed for weighing the cage could thus be hung on the balance with the animal in a horizontal position, no matter what attitude it had assumed. The purpose of this was to prevent localized increases in pressure within the coelom and the consequent rupture of the integument. Before weighing, the cages were blotted with filter-paper and care was taken to ensure that no water was trapped under the ambulacral grooves. In experiments where the animals were permitted the free range

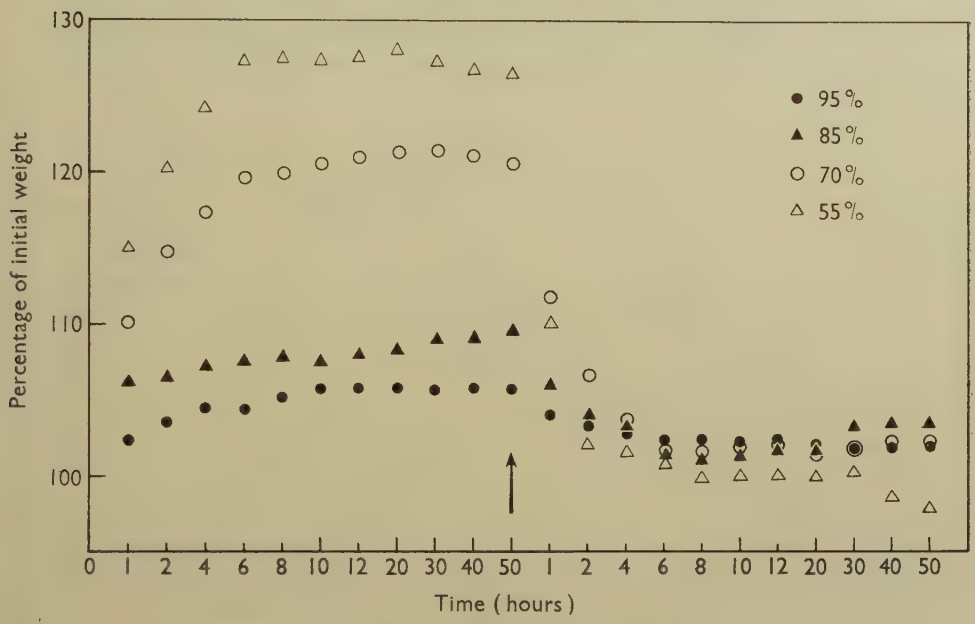

Fig. 1. Weight changes in large animals in diluted media. Arrow indicates replacement in normal sea water.

of the tanks and simply removed and dried with a cloth prior to weighing, frequent and large decreases in weight were observed. This was found invariably to be due to splitting of the integument, generally near the tips of the arms, followed by loss of coelomic fluid. For each experiment five animals of about 30 g were weighed at regular intervals, a further five being kept as controls. Four separate dilutions were employed—95, 85, 70 and 55 % sea water. After 50 h in these dilutions the animals were transferred back to normal sea water and their weight changes followed for a further 50 h. During the experiments the control animals did not vary in weight by more than 3 %.

Some small fluctuations in weight were occasionally recorded. These mostly coincided with eversions and retractions of the stomach, similar

observations being made by Maloeuf. From the graphs illustrating the time course of their behaviour in diluted media, it will be seen that there is no suggestion of a return to their original weight, and it is concluded that *A. rubens* is incapable of weight or volume regulation. This is contrary to the findings of both Bethe (1934) and Maloeuf (1938), but as neither of them mentions any special precautions taken during the weighing of their animals, and, as even quite delicate handling can cause bursting of the integument, it is not improbable that the more anomalous results of these authors may be

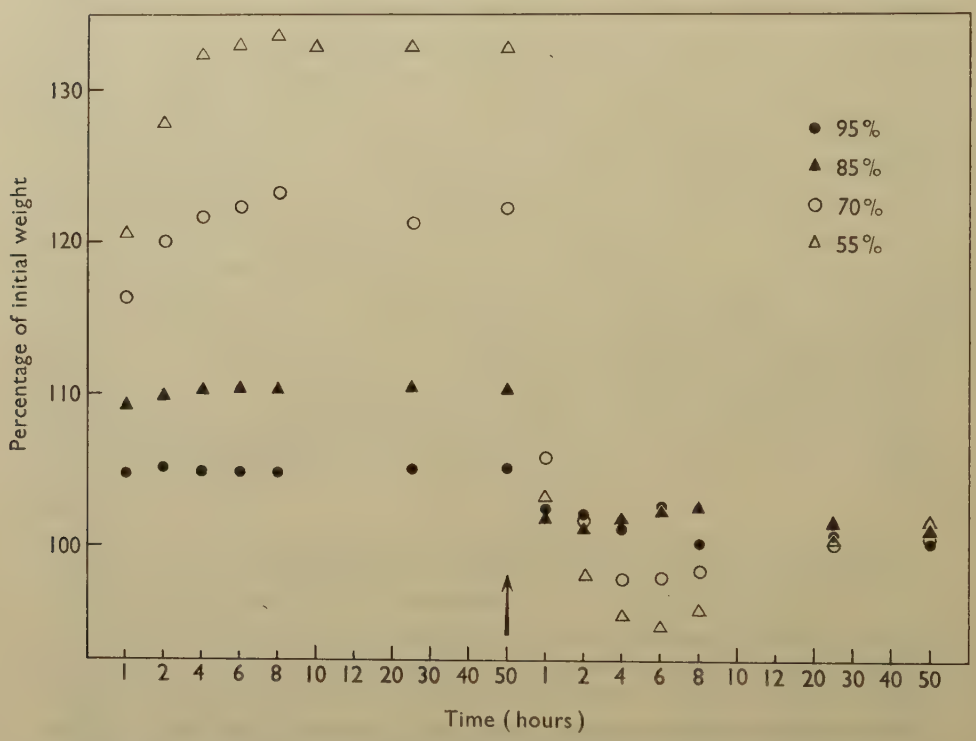

Fig. 2. Weight changes in small animals in diluted media. Arrow indicates replacement in normal sea water.

due to this cause. This series of experiments was repeated using smaller, and presumably younger, animals of between 2 and 5 g. The time course of their weight changes is illustrated in Fig. 2. In sea water more dilute than about 50%, the weight changes were less than expected. In both 30 and 20% sea water a rise of only 20% in weight was encountered and in tap water many animals actually lost weight. This is contrary to the results of Garrey (1904), who found that *A. vulgaris* became much swollen after 12 h in fresh water ($\Delta = -0{\cdot}02^\circ$ C). It is clear that at these dilutions the integument of the

animal is damaged severely, probably resulting in a rapid loss of salts, thus leading to a reduced water uptake.

In order to compare the results obtained from different animals and different experiments, it is necessary to have some standard by which to measure permeability. The reciprocal of the time taken for a 3% change in weight to occur, a purely arbitrary figure, was used for the following reasons. Such a value usually fell on the linear portion of the graph, it is a sufficiently small value for the change in the concentration difference between the inside and the outside of the animal to be negligible as also would be the changes in the volume, surface area and thickness of the integument. In Fig. 3 comparison is made between large and small animals for the transfer of water both into,

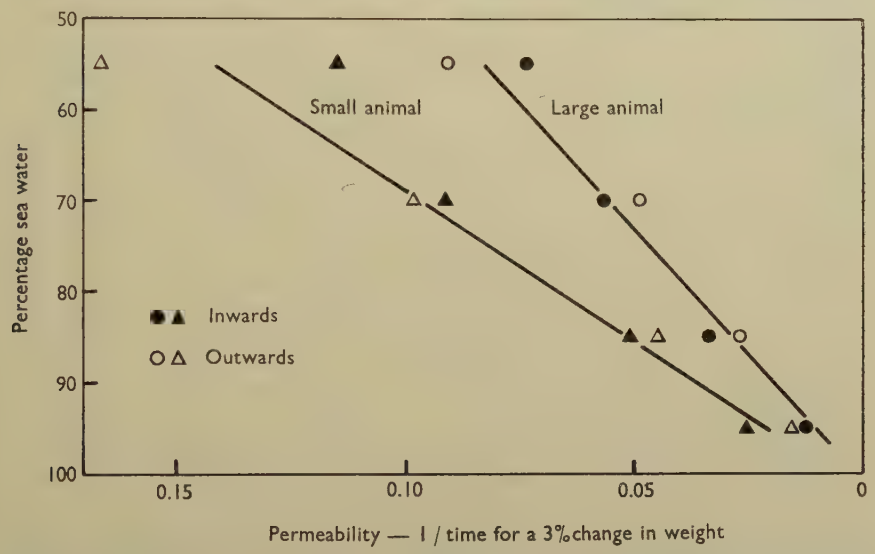

Fig. 3. Comparison of rates of water movement into and out of both large and small animals.

and out of, the animals. It will be seen that the rate of such transfer is nearly twice as great in the smaller animals. However, the total uptake by both large and small animals is very similar at each of the dilutions used and is proportional to the degree of dilution (see Fig. 4).

Direct measurement of the surface area of a starfish presents an almost impossible task due to the presence on the aboral surface of numerous dermal branchiae, and on the oral surface, the tube feet. However, some comparison between large and small animals can be made if the starfish arm be considered as a simple geometrical figure, namely a cone. Fig. 5 indicates the relationship between arm length and the (surface area)/volume ratio for some thirty animals. In the weight regulation experiments large animals had an arm length of about 5–6 cm and the small ones 1·5–2 cm. It will be seen therefore, that

relative to the body volume, the surface area of the small animals is nearly twice as great as for the large ones. It is suggested, therefore, that this factor alone could account for the increased rate of water transfer found in the smaller animals.

The effect of temperature on the rate of water uptake in large animals was investigated by examining the time course of the weight changes of five animals in a fixed dilution of sea water—70%—at 0, 5, 10, 15 and 20° C.

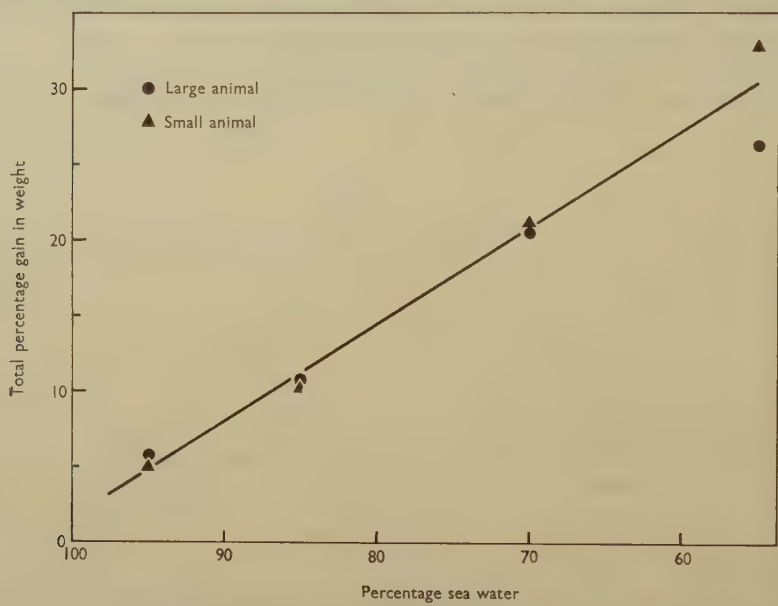

Fig. 4. Comparison of the total water uptake in large and small animals.

A further five animals were kept in normal sea water at these temperatures as controls. The same measure of permeability as before was employed, and this value is plotted against the experimental temperature in Fig. 6. Increasing the temperature increases slightly the rate of water movement, the Q_{10} being about 1·5. Furthermore, evidence of weight regulation over the experimental range of temperatures is lacking.

It is not inconceivable that some seasonal influence may affect the permeability to water. For this reason the rate of water uptake in 70% sea water was measured at monthly intervals during the year 1956/57. The permeability values so obtained are also plotted on Fig. 6 against the environmental temperature at which they were performed. As they fall within the limits of variation set by the effects of temperature, it is suggested that in so far as permeability to water is concerned, any innate seasonal difference is negligible. In none of these experiments was there any tendency for weight regulation to occur.

So far, the account has been concerned solely with the permeability of the animal as a whole, no attempt being made to assess the relative importance of the contributions made by the two major surfaces of the body, namely, the integument and the tube feet, in the transfer of water. Therefore, several attempts were made to examine the rate of water uptake with the ambulacral grooves sealed with such substances as dental cement and various plastic compounds. Unfortunately, any substance giving a good seal also resulted in

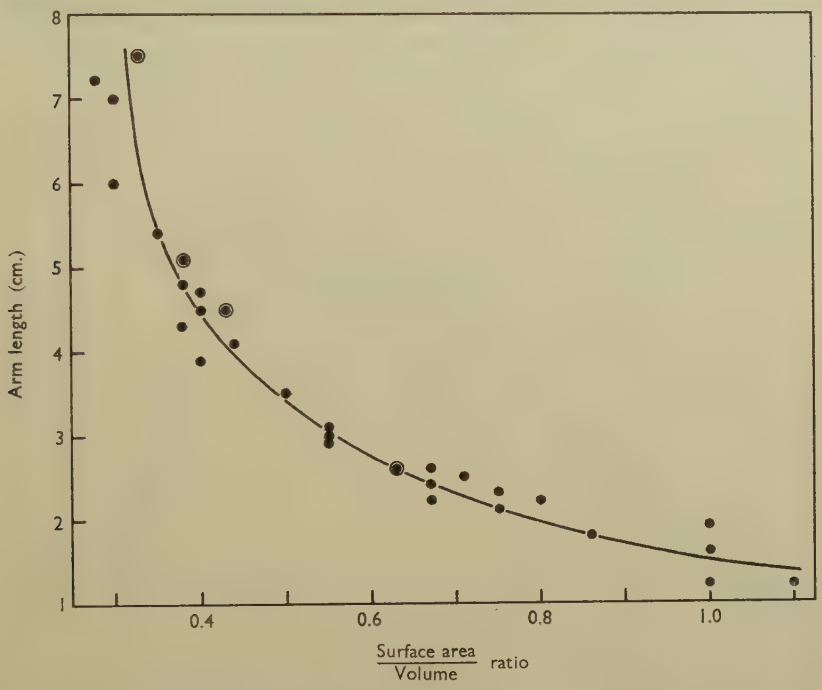

Fig. 5. Relationship between arm length and the (surface area)/volume ratio for a range of animals.

the splitting of the integument at its junction with the sealing compound, so no reliable measurements were possible. Some estimate of the relative permeability of the two surfaces was, however, obtained by measuring the loss in weight due to evaporation when either the aboral or the oral surface was exposed to the atmosphere. For this purpose a bed of dry gravel was used with twenty animals exposed in each of the positions mentioned. After 3 h five animals from each group were removed, weighed and the chloride concentration in both the perivisceral and water vascular cavities estimated by the method of Milton & Waters (1949). A further five animals from each group were replaced in normal sea water and weighed at intervals. After 18 h they had fully recovered. Similar estimations were made after a 6 h period

of exposure, but on this occasion there was no recovery in sea water. Table 1 shows the values obtained and it suggests that the amount of water lost was independent of the surface exposed, furthermore, the chloride concentration was increased by a similar amount in both coelomic cavities. Bearing in mind

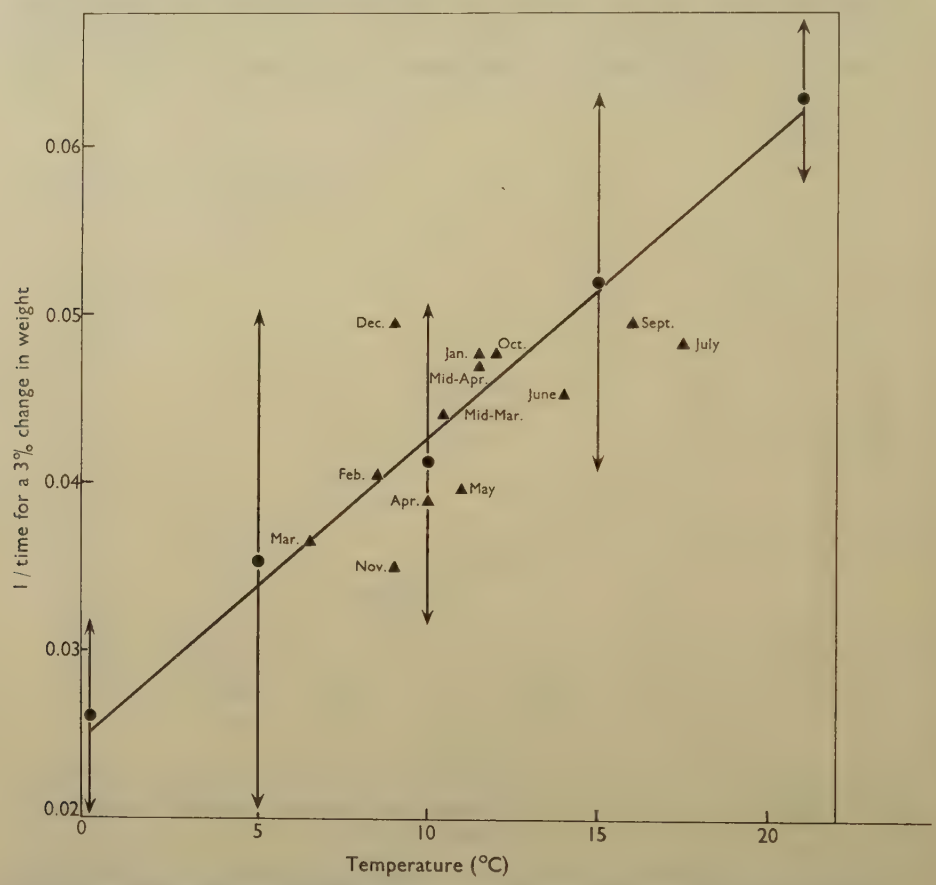

Fig. 6. Effect of temperature and season on the rate of water transfer. Arrows indicate the standard deviation due to the effects of temperature.

TABLE 1. RATES OF WATER LOSS AND CHLORIDE CONCENTRATION IN BODY FLUIDS OF EXPOSED ANIMALS

	Aboral surface uppermost			Oral surface uppermost		
		Increase in Cl^- conc. in fluids (%)			Increase in Cl^- conc. in fluids (%)	
Time of exposure	Loss in weight (%)	PVC	Amb.	Loss in weight (%)	PVC	Amb.
After 3 h	13	19·4	23·5	11	22·0	20·6
After 6 h	20	26·0	28·4	21	28·4	26·6

the relative volumes of these two cavities (Smith, 1946), it would be necessary for water to pass into the ampullae from the perivisceral cavity in order to permit such a large quantity to be lost via the ambulacral surface.

In order to examine the permeability of the various structures present in the starfish, isolated preparations of the tube feet, tube foot ampulla systems, stomach and gut caeca were made. The tube feet were ligatured in the retracted condition, for otherwise bursting was often encountered when they were placed in diluted media, which for this series of experiments was 70% sea water at 15° C. As the weight changes were very rapid compared with

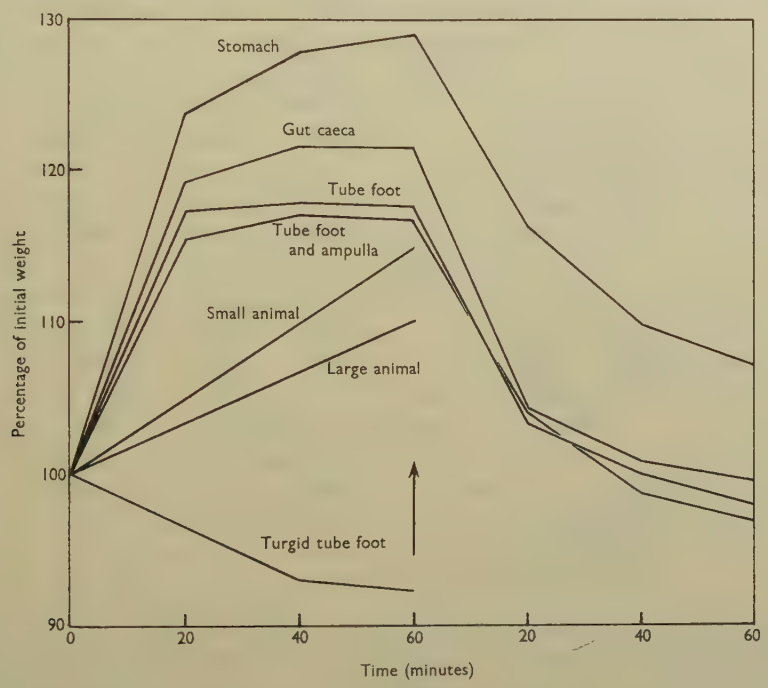

Fig. 7. Weight changes in isolated preparations from *Asterias rubens* in 70% sea water. Arrow indicates replacement in normal sea water.

those in the intact animal, weighings were made at 20 min intervals for 1 h before returning the preparation to normal sea water for a similar period. The osmotically inactive material, in the form of adherent ossicles in the case of the tube-foot ampulla systems, was removed after the experiment and the weight thus allowed for when calculating the percentage changes in weight. The results of this experiment, shown in Fig. 7, indicate that the tube feet, ampullae, stomach and gut caeca are all extremely permeable to water and therefore a movement of water from one cavity to another as suggested above is quite feasible.

Finally, osmotic pressure measurements were made on the perivisceral and ambulacral fluids of animals living under environmental conditions and upon those which had reached weight equilibrium in diluted media. Both freshly drawn and centrifuged samples were employed, but no difference was to be found between them. The method used was that of Freeman & Rigler (1957) based upon the melting times of the samples and a number of

TABLE 2. OSMOTIC PRESSURE OF THE BODY FLUIDS UNDER ENVIRONMENTAL AND EXPERIMENTAL CONDITIONS

Fluid	Value (°C)	S.D. (°C)	No. of animals
(i) Melting-time method			
Sea water	−1·90	0·04	10
Perivisceral fluid (freshly drawn)	−1·88	0·04	5
Perivisceral fluid (centrifuged)	−1·86	0·05	10
Ambulacral fluid (freshly drawn)	−1·86	0·05	5
Ambulacral fluid (centrifuged)	−1·92	0·03	10
Animals equilibrated in 70% sea water			
70% sea water	−1·34	0·06	5
Perivisceral fluid (freshly drawn)	−1·34	0·06	5
Ambulacral fluid (freshly drawn)	−1·36	0·05	5
(ii) Baldes–Hill method			
	(mosmoles)	S.D.	
Sea water	1065	12	6
Ambulacral fluid (centrifuged)	1057	8	24
Ambulacral fluid (from single ampullae)	1065	30	6

standards as originally described by Jones (1941) and Gross (1954). Later, a modification of the Baldes–Hill thermoelectric method was employed. The values so obtained are presented in Table II and merely confirm earlier work. Both the perivisceral and ambulacral fluids of *A. rubens* are isotonic with the environment and the animal is incapable of any osmoregulation in either coelomic cavity should the environment become diluted.

DISCUSSION

A. forbesi (Desor) has a lower limit of acclimatization of about 17‰ (Loosanoff, 1945) and can withstand temperatures of up to 33·5° C (Huntsman & Sparks, 1924). Lee (1941) also notes that *A. forbesi* cannot endure a salinity below 18‰ for very long. *A. vulgaris* (Verrill) is by comparison less hardy, and hence is physiologically at least more like our own *A. rubens*. G. Smith (1940) found that this latter American species could live for a short

while in water of salinity 14‰, but this experiment was criticized by Loosanoff as being of too short a duration for a true value for the lower limit of acclimatization to be obtained. In a more detailed study Topping & Fuller (1942) arrived at a figure of about 22‰ and backed this with field observations in the Narraguagus estuary (Maine, U.S.A.) where they found that its distribution ceased when the low tide salinity fell below 27·4‰. Furthermore, it seems unable to withstand temperatures in excess of about 25° C, similar to the maximum for *A. rubens*. If physiological differences such as these can exist in separate species of *Asterias*, then it seems reasonable to suppose that such differences as have been found between the North Sea and Baltic Sea populations of *A. rubens* might be justifiable grounds for postulating that they belong to different physiological races. The value for the lower limit of salinity tolerance obtained here for the North Sea *A. rubens* of around 23‰ must be viewed with some caution. No experiments were of a sufficient duration to test whether the animals could mature and breed at this dilution. Sagara & Ino (1954) showed that the bipinnaria of the Japanese starfish *A. amurensis* (Lutken) could tolerate a salinity of 13‰. However, even if the tolerance of the larva of *A. rubens* is greater than the adult, the value already obtained for the adult will still be a factor limiting its distribution. Even so, there is a considerable gap between a value of 23‰ and the 8‰ at which the Baltic Sea population can live and reproduce, although the 'spawning intensity' of animals in this locality is much reduced and asteroid larvae seem very sensitive to reduced salinity (Thorson, 1946). In addition to differences in salinity tolerance, the gross chemical composition, size of ova at maturity, breeding cycle and reaction times for the 'righting process', have also been shown to differ (Bock & Schlieper, 1953; Kowalski, 1955; Schlieper, 1957).

It is now stated in certain textbooks (e.g. Prosser *et al.* 1950) upon the authority of Bethe (1934) and Maloeuf (1938), that *A. rubens* is capable of regulating its weight in dilute media. In the present investigation nearly 300 animals have been examined and in no instance was there the slightest tendency for them to regulate their weight, even after as long as 50 h in diluted media. Furthermore, this situation is uninfluenced by temperature, season and size.

It was not possible to obtain a direct estimate of the contributions made by the oral and the aboral surfaces in the uptake of water when the animal is in a diluted medium. Rates of evaporation, however, suggest that there is very little difference between the two. When stranded, *A. rubens* is nearly always found with the oral surface against the substratum. In many instances it was discovered that the stomach was everted and penetrated some little distance down between the gravel. At the lower levels of the beach, where the animal is most frequently to be found, the water table is usually only a few centimetres below the surface and it is feasible that water evaporated from the surface of the animal can be replaced osmotically from this source.

Eversion of the stomach seems primarily to be due to oxygen lack, but may thus also be effective in preventing desiccation. Analysis of the body fluids of a few stranded animals indicated that they were only slightly more concentrated than the sea water. Animals stranded for any length of time on dry gravel seem invariably to be desiccated. Meyer (1935) found that each surface contributed equally to the total oxygen uptake. Experiments involving the weight changes of isolated preparations in diluted sea water confirmed that the bounding membranes of the animal are virtually freely permeable to water. Measurements of osmotic pressure under environmental and experimental conditions indicated that the animal is incapable of any osmotic regulation, and this evidence taken in conjunction with the permeability studies suggests that the two main coelomic cavities of the starfish behave, osmotically, as one, and that differences induced in one are reflected in the other.

This work was carried out during the tenure of a Research Assistantship at Sir John Cass College, London. My thanks are due to Professor J. E. Smith, F.R.S., for his supervision of the thesis of which this paper forms a part, and for the use of his Department's Marine Laboratory at Whitstable. I am also grateful to the Director and Staff of the Plymouth Marine Biological Laboratory for their hospitality whilst I occupied the London and British Association Tables there. Part of the travelling expenses were defrayed by a grant from the Central Research Fund of London University.

SUMMARY

Evidence is presented to suggest that the salinity tolerance of the adult North Sea *Asterias rubens* is around 23‰, whereas that of the Baltic Sea animals is 8‰. This, together with other evidence outlined, is thought to be sufficient reason to justify the use of the term 'physiological race' where these two populations are concerned.

Adult *A. rubens* from the North Sea is incapable of any degree of weight regulation in diluted media within the ecological temperature range, size or state of the breeding cycle.

Similarly, it is incapable of any osmotic regulation and it is suggested that the two main coelomic cavities constitute an osmotic unity.

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PERCHLORATE IN SEA WATER

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(Text-figs. 1–5)

In the perchlorate ion four oxygen atoms are arranged tetrahedrally about a central chlorine atom. The symmetry of this configuration and the fact that the chlorine atom has all its valency electrons shared, and is surrounded on all sides by oxygen atoms, confers great stability on the perchlorates compared with the much less stable chlorates. Many perchlorates are stable at temperatures of up to 500° C. The oxidizing properties exhibited by hot concentrated perchloric acid have been attributed to the undissociated acid. When cold, or in its dilute solutions it is completely dissociated and free from oxidizing properties. Under these conditions the perchlorate ion is only reduced by the strongest reducing agents such as Cr^{2+} and Mo^{3+}.

Not only are perchlorates difficult to reduce but they are also only formed in comparatively few reactions. The only recorded modes of formation are based on the action of heat on chlorates, electrolytic oxidation of chloride, probably via chlorate, and chemical or photochemical oxidation of chlorate.

Perchlorates are known to occur naturally only in the nitrate beds of Chile, which average 0·5% ClO_4^- but may contain up to 6% ClO_4^-. In these beds perchlorate is associated with ions of high redox potential such as iodate, chromate and selenate. The mode of formation of these ions is not known, but it is believed that they were not formed *in situ*.

Recently Baas Becking, Haldane & Izard (1958) claimed to have detected up to 150 p.p.m. of perchlorate in ocean water, even more in some estuarine waters, and up to 10,000 p.p.m. in brines. They consider that this perchlorate is formed from chloride by an unspecified oxidation mechanism involving iodate. They further state that sea-water samples should be stored under conditions of high redox potential to prevent reduction of perchlorate to chloride. Complete reduction of 150 p.p.m. of ClO_4^- in a sea water would increase its chlorinity by *ca.* 0·053‰. Perchlorate formation and reduction would thus have a serious effect on the use of chlorinity as a conservative property of sea water and on its use for computing σ_t values.

In view of these facts it seemed important to have an independent check on the amount of perchlorate present in sea water; particularly because of the inherent improbability that the perchlorate ion would be reduced to chloride under conditions normally used for the storage of sea water samples.

Baas Becking *et al.* (1958) describe five methods for the detection and

determination of traces of perchlorate in water. They themselves apparently used a method based on the precipitation of methylene blue perchlorate. The precipitated perchlorate was separated by filtration or centrifugation, and the excess methylene blue was determined colorimetrically in the clear liquid. They state that the sensitivity of this method is 100 p.p.m. ClO_4^-. Such a technique tends to give high results owing to chloride interference and to the ease with which methylene blue is adsorbed by glass. In a further method which they describe, methylene blue perchlorate was extracted with *iso*-propyl methyl ketone and then determined colorimetrically in the organic phase. They claim a sensitivity of 1 p.p.m. for this procedure. Unfortunately, although they do not state this, chloride is also extracted, and erroneously high results are obtained if appreciable amounts of chloride are present.

It is thus evident that the use of methylene blue should be avoided in any confirmatory work on perchlorate in sea water. A review of the literature revealed that, although the perchlorate ion exhibits very few specific reactions, it does form comparatively insoluble perchlorates with a number of organic compounds, such as strychnine (Denigès, 1917), the ferrous sulphate complex of *o*-phenanthroline (Smith & Richter, 1944), nitron (Loebich, 1926), tetraphenylarsonium chloride (Willard & Smith, 1939).

The perchlorates of strychnine and of ferrous *o*-phenanthroline are rather too soluble to be used for the detection of traces of perchlorate and were not investigated. Experiments were made using nitron and good recoveries of 100 p.p.m. of perchlorate from distilled water were obtained. In solutions containing chloride a gelatinous precipitate of nitron chloride was formed and this method was not studied further.

The use of tetraphenylarsonium chloride, $(C_6H_5)_4As\,Cl$, was next investigated. It was found that this reagent gave no precipitate with distilled water, perchlorate-free sodium chloride solution or filtered sea water. In the presence of 5 p.p.m. of added ClO_4^-, each gave a visible precipitate of tetraphenylarsonium perchlorate within 10 min. of the addition of the reagent. It therefore appeared that if perchlorate was present in sea water its concentration must be below 5 p.p.m.

Since 5 p.p.m. of perchlorate is the lowest concentration that can be directly detected with this reagent, it was necessary to concentrate the perchlorate before lower concentrations than this could be determined. This was achieved by adding a known amount of potassium perrhenate to the water before addition of the reagent. The precipitate of the analogous tetraphenylarsonium perrhenate co-precipitated tetraphenylarsonium perchlorate quantitatively.

The concentration of the perchlorate in the mixed precipitate was determined by infra-red spectrophotometry. The two compounds have similar absorption characteristics over most of the spectrum from $5{\cdot}5\,\mu$ to $15\,\mu$ (Fig. 1), since the organic moiety is the same in both. Their Cl-O and Re-O vibrations give rise to strong widely separated absorption bands, at $9{\cdot}13$ and $11{\cdot}0\,\mu$,

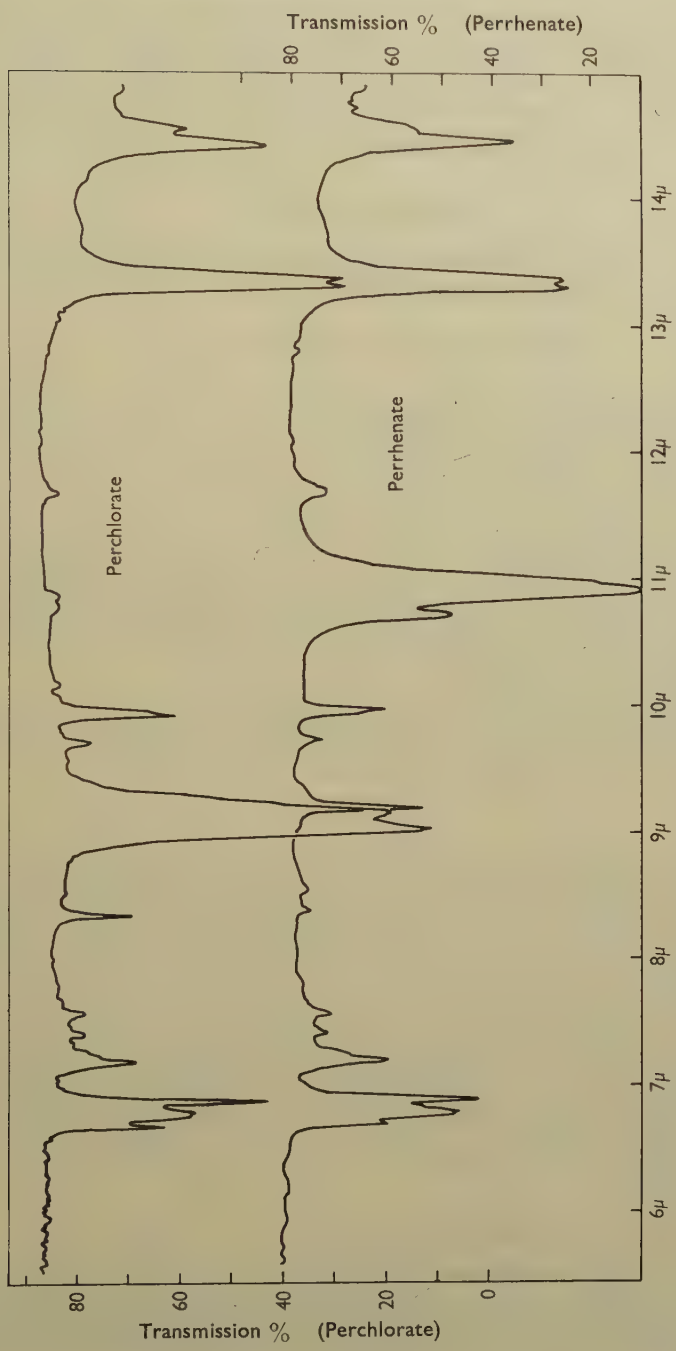

Fig. 1. Transmission-wavelength curves of tetraphenylarsonium compounds (Nujol mull).

respectively. At the wavelength of 9·13 μ which was used for the determination of perchlorate, the absorption due to the perrhenate compound was negligible. The latter also has a weak absorption maximum at 9·25 μ, but this caused no interference with measurement at the 9·13 μ maximum if a spectrophotometer of good resolving power was used.

EXPERIMENTAL

A model 21 Perkin Elmer infra-red spectrophotometer with NaCl optics was used in all the work recorded below.

Determination of perchlorate in sea water

Reagents

(1) Potassium-perrhenate solution.

Dissolve 0·1200 g of pure potassium perrhenate in water and dilute to 100 ml.

(2) Tetraphenylarsonium chloride reagent.

Dissolve 0·40 g of tetraphenylarsonium chloride in about 40 ml. of water, filter through a retentive filter paper and dilute to 100 ml.

(3) Perchlorate-free 3·5 % sodium chloride solution.

Heat 40 g of sodium chloride to 700° C in a platinum dish for 30 min. Prepare an aqueous solution containing 35 g of this salt per litre. Although perchlorate has not yet been detected in any batches of A.R. sodium chloride, it has been thought advisable to heat it to 700° C to destroy any possible traces of perchlorate, as this solution is used for the determination of the reagent blank.

(4) Standard perchlorate solutions.

Prepare a solution containing 1·393 g of potassium perchlorate/l., this stock solution contains 1·0 mg ClO_4^-/ml. The working solution is prepared by diluting 1 ml. of this solution to 100 ml.; it contains 10 μg ClO_4^-/ml.

Method

Place 100 ml. of filtered sea water in a 250 ml. conical flask, add 5 ml. of potassium perrhenate solution and 5 ml. of tetraphenylarsonium chloride reagent and mix well. Allow the covered flask to stand overnight in a refrigerator at *ca.* 2° C. Filter off the crystalline precipitate on a 7 mm diameter Whatman No. 52 filter-paper fitted in a small Hirsch funnel. Wash well with ice-cold water (20 ml.). Dry *in vacuo* at room temperature for several hours. Prepare a potassium bromide disc containing 4 % of the dried tetraphenylarsonium derivative. Measure its infra-red absorption over the wavelength range 8·8–9·5 μ. Determine the intensity of absorption by measuring the area contained under the 9·13 μ absorption band (Phillpots & Maddams, 1960). Carry out a blank determination in the same manner using 100 ml. of 3·5 % sodium chloride solution. Calibrate the method using 100 ml. portions of 3·5 % sodium chloride solution, to which aliquots of the working standard perchlorate solution have been added.

Sensitivity and reproducibility of method

Various amounts of perchlorate in 100 ml. aliquots of 3·5 % sodium chloride solution (concentrations of 0·02–2·16 p.p.m. ClO_4^-) were examined as described above. The resultant absorption curves over the range 8·8–9·5 μ are shown in Fig. 2. The optical densities computed from the area under the 9·13 μ absorption band show a

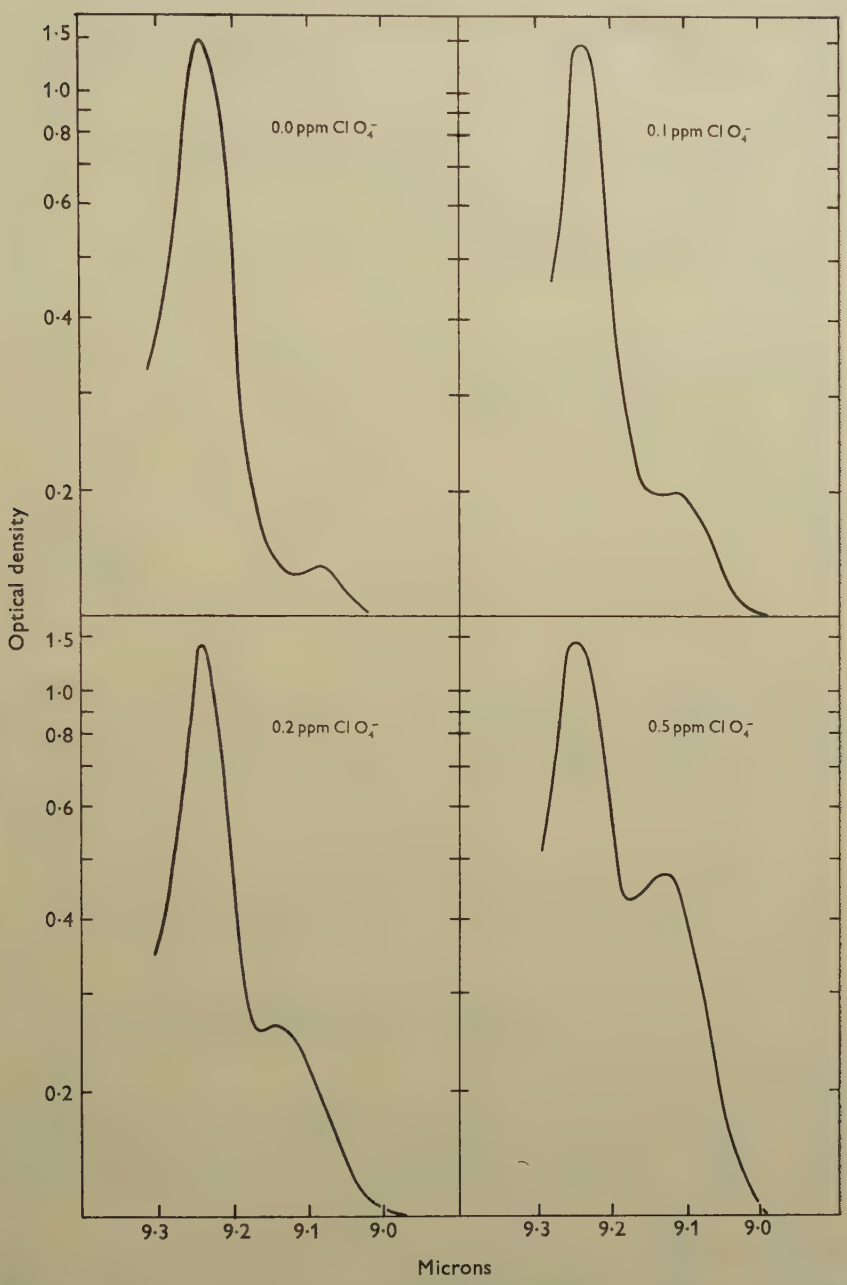

Fig. 2. Absorbance-wavelength curves of tetraphenylarsonium perrhenate, precipitated from sea water containing various concentrations of perchlorate ion.

linear relationship to perchlorate concentration (Fig. 3) over this range of concentrations. It is estimated that the minimum detectable perchlorate concentration is 0·03 p.p.m. In order to evaluate the reproducibility of the method, replicate determinations were made on 100 ml. aliquots of two sodium chloride solutions, containing 0·13 and 0·65 p.p.m. ClO_4^-, these gave coefficients of variation of 10·0 and 9·1 % respectively (six determinations in each case). Within the limits of experimental error the method gave the same recovery of perchlorate from sea water and distilled water as from 3·5 % sodium chloride solution.

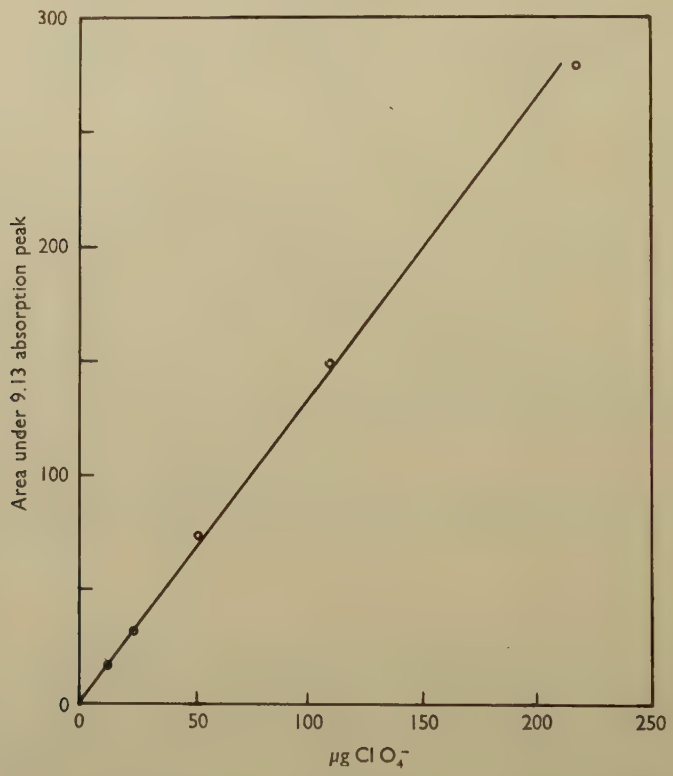

Fig. 3. Calibration curve for determination of perchlorate. (Perchlorate in μg related to area under 9.13μ absorption peak.)

Interferences

Tests showed that none of the normal constituents of sea water interfered with the method, when present in their usual concentrations. The possible interference of other oxy-halogen acids was investigated, and it was found that no interference was experienced with 300 p.p.m. ClO_3^- or IO_3^- or with 2 p.p.m. IO_4^-.

Storage of samples

There was no detectable change in the perchlorate content of sea water samples, which had been enriched with 1 p.p.m. of perchlorate, on storage for about 1 month in full glass or polythene bottles. Furthermore no bio-reduction of perchlorate occurred

in perchlorate-enriched sea waters in which cultures of micro-organisms were growing freely. These findings which agree with what is known about the difficulty of reducing the perchlorate ion, directly confute the statement of Baas Becking *et al.* (1958) that perchlorate is reduced to chloride under conditions of slightly low redox potential.

Solubility of tetraphenylarsonium perchlorate and perrhenate

The solubilities of the tetraphenylarsonium derivatives in water, 3·5% sodium chloride solution and sea water were determined. The optical densities of saturated solutions, prepared from the purified salts, were measured at 264 mμ* and compared with those of solutions of the two compounds of known strength. The results are shown in Table 1.

TABLE 1. SOLUBILITIES OF TETRAPHENYLARSONIUM COMPOUNDS IN WATER, 3·5% SODIUM CHLORIDE SOLUTION, AND SEA WATER

	Solubility (mg/l.)					
	Water		3·5% sodium chloride		Sea water (Cl = 18·97‰)	
Temperature (°C) ...	21·0	2·5	21·0	2·5	21·0	2·5
$(C_6H_5)_4AsClO_4$	20·2	16·3	32·1	22·0	37·0	27·8
$(C_6H_5)_4AsReO_4$	24·9	19·6	35·7	23·0	36·1	25·9

RESULTS

Analyses for perchlorate were made on samples of surface waters from twenty-six stations in the North Atlantic, English Channel, Irish Sea, Barents Sea, Mediterranean Sea, Red Sea, Arabian Sea, Persian Gulf and Indian Ocean (Table 2). In addition analyses were made of the deep waters at seven of these stations.

Although the analytical method will detect 0·03 p.p.m. of perchlorate in sea water, in no case was any found. These negative results are supported by the observation of Klemperer (1957) that the addition of as little as 0·05 p.p.m. of perchlorate to sea water reduced the rate of uptake of ^{131}I by *Fucus ceranoides* by 60%. Shaw & Klemperer (private communication) have found a similar effect with *F. serratus*.

It appears therefore that the high concentrations of perchlorate found in sea water by Baas Becking *et al.* are completely erroneous. It is likely that this error was caused by the interference of chloride in their analytical method (*vide supra*), and it seems significant that the amount of perchlorate allegedly present in their samples is generally roughly in proportion to their chloride content.

It was thought that if there was any perchlorate present in sea water, it might have been formed, *in situ*, by photochemical oxidation by hydroxyl

* Aqueous solutions of both derivatives have their absorption maxima at 220 mμ with a lesser maximum at 264 mμ.

radicals formed by the catalytic action of traces of transition metals. Experiments were therefore carried out in which 100 ml. aliquots of 3·5 % sodium chloride solutions, buffered to pH 8·1 with sodium carbonate–bicarbonate buffer, and containing 0·1 p.p.m. of Fe^{3+}, Ni^{2+}, Co^{2+}, Tl^{+} or Cu^{2+}, were irradiated at 30° C in silica flasks for 48 h, close to a mercury vapour lamp with quartz burner. In no case was any detectable perchlorate formed. Since the extremely energetic 2536 Å mercury radiation does not produce any

TABLE 2. SAMPLING STATIONS FOR DETERMINATION OF PERCHLORATE

Stn. no.	Lat.	Long.	Sampling depths (m)	Cl ‰
		Barents Sea		
F3	74° 00′ N.	20° 00′ E.	0	18·674
F4	74° 25′ N.	14° 15′ E.	0	19·479
		North Atlantic		
F1	65° 00′ N.	05° 00′ E.	0	19·537
H219	62° 30′ N.	14° 30′ W.	400, 1000	19·556, 19·580
30	48° 40′ N.	06° 45′ W.	0	19·614
26	46° 41′ N.	10° 20′ W.	0	19·766
25	44° 01′ N.	07° 48′ W.	0	19·710
23	40° 33′ N.	09° 35′ W.	0	19·619
20	36° 56′ N.	00° 05′ W.	0	19·853
Cavall	46° 30′ N.	08° 00′ W.	0, 511, 956	19·399, 19·347, 19·354
Cavall	46° 30′ N.	08° 00′ W.	2082, 2987, 3855	19·738, 19·808, 19·702
		South Atlantic		
B1	36° 49′ S.	21° 18′ E.	0	19·572
		Irish Sea		
Kish	53° 20′ N.	05° 17′ W.	0	19·069
		Mediterranean		
16	36° 31′ N.	00° 40′ E.	0	20·465
M1	42° 00′ N.	06° 00′ E.	1, 200, 1000	21·233, 21·302, 21·256
M3	42° 58′ N.	08° 14′ E.	0, 2500	21·126, 21·266
13	38° 08′ N.	09° 54′ E.	0	20·965
12	33° 01′ N.	28° 38′ E.	0	21·382
E9	33° 01′ N.	28° 32′ E.	0	21·361
		Red Sea		
E6	20° 00′ N.	38° 25′ E.	0, 1000	21·464, 22·487
		Arabian Sea		
7	17° 00′ N.	57° 02′ E.	0	19·993
2	23° 06′ N.	64° 02′ E.	0	19·979
E3	22° 31′ N.	61° 55′ E.	3000	19·246
		Persian Gulf		
1	26° 00′ N.	55° 00′ E.	0	20·597
		Indian Ocean		
B2	50° 02′ S.	35° 12′ E.	0	18·757
B4 (1)	30° 07′ S.	64° 14′ E.	0	19·745
B4 (2)	30° 21′ S.	67° 15′ E.	4720	19·221
B6 (1)	33° 31′ S.	101° 08′ E.	0	19·843
B6 (2)	36° 08′ S.	106° 13′ E.	998	19·038
B6 (3)	33° 31′ S.	101° 07′ E.	6510	19·209

detectable amount of perchlorate from chloride ion, it is unlikely that any will be formed naturally by the action of sunlight, as this contains very little radiation of wavelength shorter than 3200 Å.

Investigation of the effect of perchlorate on marine organisms

Before it was realized that the perchlorate content of sea water was much less than that claimed by Baas Becking *et al.*, experiments were initiated to investigate the effect of the ion on marine organisms. The organisms selected were various unicellular planktonic algae, which are usually quite sensitive to the presence of toxic substances.

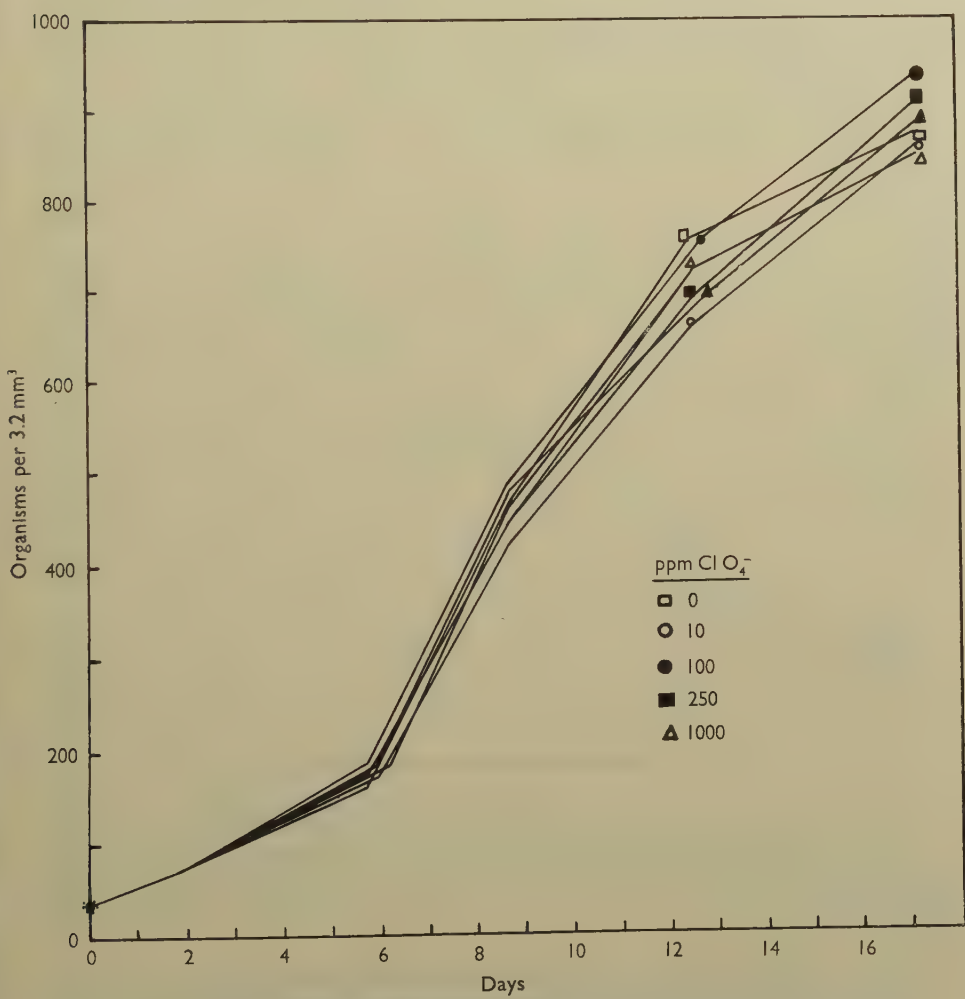

Fig. 4 Effect of perchlorate ion on growth of bacteria-free *Phaeodactylum tricornutum*.

Bulk cultures (2 l.) of the organisms (mainly bacteria-free) were prepared under sterile conditions in the medium described by Droop (1958). After several days, when the organisms were growing freely, aliquots (300 ml.) of these cultures were placed in sterilized conical flasks, containing varying volumes of a solution of chlorate-free sodium perchlorate in sterilized sea water. Sterilized sea water was added to bring the final volume to 400 ml. The final perchlorate concentrations were 10, 100, 250 and 1000 p.p.m. ClO_4^-.

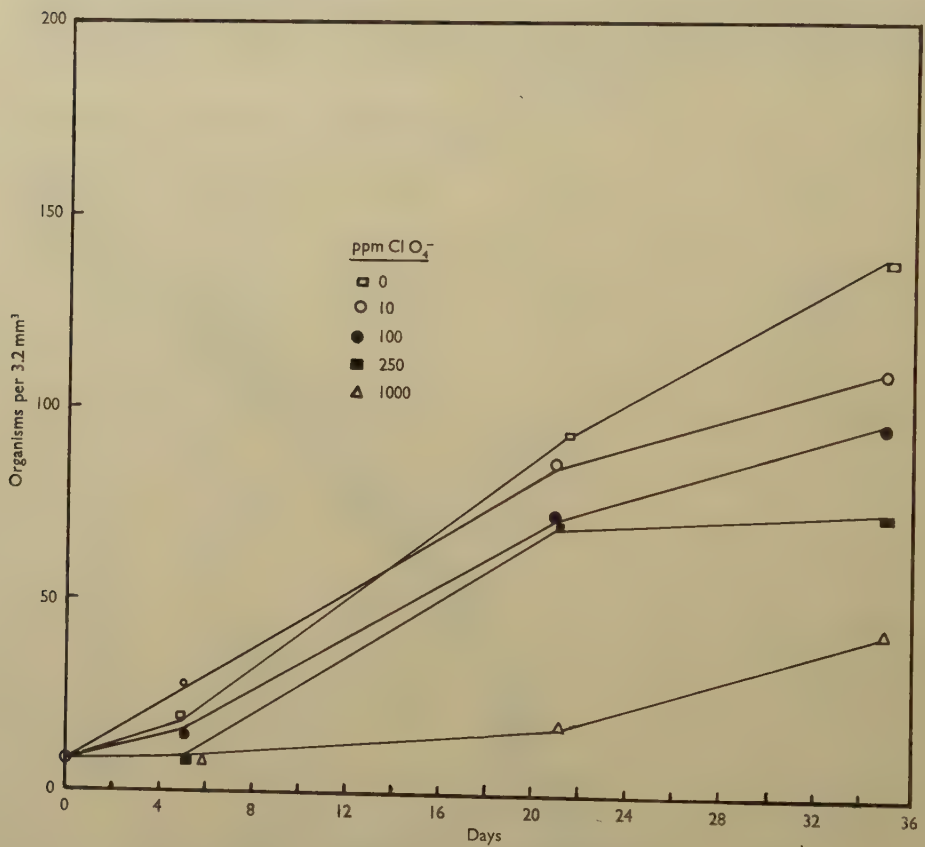

Fig. 5. Effect of perchlorate ion on growth of *Gymnodinium vitiligo*.

A control was also prepared consisting of 300 ml. of the same culture with 100 ml. of sterilized sea water. The cultures were grown at 22° C, illuminated by a fluorescent lamp. The rate of growth of the organisms was determined at intervals by cell counting or by measurement of the optical density of the culture as appropriate.

Of the organisms investigated *Phaeodactylum tricornutum*, *Monochrysis lutheri*, *Isochrysis galbana*, and *Nannochloris oculata* were completely resistant

to even 1000 p.p.m. of perchlorate, as typified by *P. tricornutum* (Fig. 4). Some organisms (viz. *Prorocentrum micans* (St German's strain), *Gymnodinium vitiligo*, and *Peridinium trochoideum*) were more sensitive, and with 1000 p.p.m. ClO_4^- exhibited a reduced rate of growth. Certain of these at this perchlorate level also showed an induction period before growth commenced (*P. trochoideum* and *G. vitiligo*) (Fig. 5).

These observations show that the toxicity of the perchlorate ion is very slight, compared with the chlorate ion which is toxic to plant life at a level of a few parts per million. This is probably due to its great chemical stability and its resistance to bio-reduction.

The authors wish to thank Capt. G. S. Ritchie, D.S.C., R.N. and the crew of H.M.S. 'Dalrymple' for the collection of many of the sea-water samples. They are indebted to Dr Mary Parke and Dr M. R. Droop for providing marine organisms and for advice, and to Mr J. Berkeley for carrying out the infra-red measurements.

SUMMARY

The use of tetraphenylarsonium chloride for the detection and determination of perchlorate in sea water has been investigated. An aqueous solution of this reagent gives a visible precipitate of tetraphenylarsonium perchlorate with sea water containing 5 μg ClO_4^-/ml. within 10 min. For the quantitative determination of traces of perchlorate, potassium perrhenate is added to the water sample, and the tetraphenylarsonium perrhenate, which is precipitated by addition of the reagent, co-precipitates tetraphenylarsonium perchlorate. Perchlorate is determined in the mixed precipitate by infra-red absorption spectrophotometry. Using 100 ml. of sea water, a sensitivity of 0·03 μg ClO_4^-/ml. can be attained, with a coefficient of variation of *ca.* 10%. No interference is caused by the ions normally present in sea water.

Over thirty surface and deep water samples from the seas of both the northern and southern hemispheres have been examined for perchlorates, in all cases with negative results. The finding of up to 100 p.p.m. of ClO_4^- in sea water by Baas Becking *et al.* is attributed to the interference of chloride in their analytical method. Contrary to their statement, perchlorate added to sea water is not reduced under normal conditions of storage, nor does it seem to be reduced by biological processes.

The action of perchlorate on the growth of seven unicellular algae has been examined and it has been found that even 1000 p.p.m. of the ion was without action on most of them.

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THE BREEDING AND DEVELOPMENT OF *STYELA MAMMICULATA* CARLISLE

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(Text-fig. 1)

Styela mammiculata was discovered in Plymouth Sound in 1953 (Carlisle, 1954). It has become established there, although only locally abundant, and has recently been recorded from the Hampshire coast (Houghton & Millar, 1960). The adult form is solitary and stalked, clearly assignable to the genus *Styela*. Millar (1960)[1] believes that it is identical with *S. clava* Herdman. The development and larval structure of ascidians are of sufficient phylogenetic importance, concerning the relationships of genera and families, to merit a brief description. Other observations reported here may be of some value in assessing the geographic range of this species.

The breeding season is not known with certainty. Specimens failed to spawn in aquaria during October to December 1957, and July 1960. Fertile eggs were obtained by dissecting specimens of all sizes during late July 1960. This is taken to be immediately prior to a breeding season, possibly extending through August and September. By analogy to the local summer-breeding ascidians (Berrill, 1950; Marine Biological Association, 1957), *S. mammiculata* is inferred to be a boreal species, whose potential range of distribution lies mainly to the south of the English Channel, but which may prove capable of spreading farther north round the British coasts. The branchial baskets and oesophagi of forty-seven specimens were searched for commensal copepods, in the vain hope of establishing the region from which the Plymouth population originated. Only one copepod was found, a non-ovigerous female which R. V. Gotto (of this department) considers to be *Paranthessius cynthiae* (Brian).

Although natural spawning has not been observed, the anatomy of *S. mammiculata* shows it to be oviparous. Fertile eggs are 155 μ in diameter. Yellow test cells (12 μ diameter) form an incomplete layer within the thin but tough chorion, which is covered by a complete layer of clear follicle cells (20 μ diameter). The egg is rendered opaque by grey yolk and a cortical yellow

[1] Millar's article, providing the evidence that *S. mammiculata* and *S. clava* are identical appeared while this report was in proof. My inference that *S. mammiculata* is a boreal species is consonant with it being native to Japanese waters. It would be particularly interesting to learn if *Paranthessius cynthiae* occurs in Japanese specimens of *S. clava*, although the host-specificity of this copepod is unknown.

pigment. The majority of eggs obtained by dissection were immature. The most successful fertilizations were obtained by mixing the gametes of several specimens (50–90 % of the fertile eggs were self-sterile) and by adding alkali to increase sperm activity. Fertilization is followed by the maturation divisions and the formation of a yellow crescent. The pattern of cleavage and segregation of coloured plasms are virtually identical with those of *S. partita* (see Conklin, 1905), nicely illustrating the mesodermal fate maps obtained by the more accurate method of marking blastomeres with chalk grains (Ortolani, 1955). The yellow colour forms a complete crescent (presumptive muscle), the presumptive mesenchyme being restricted to two colourless patches (Fig. 1 A).

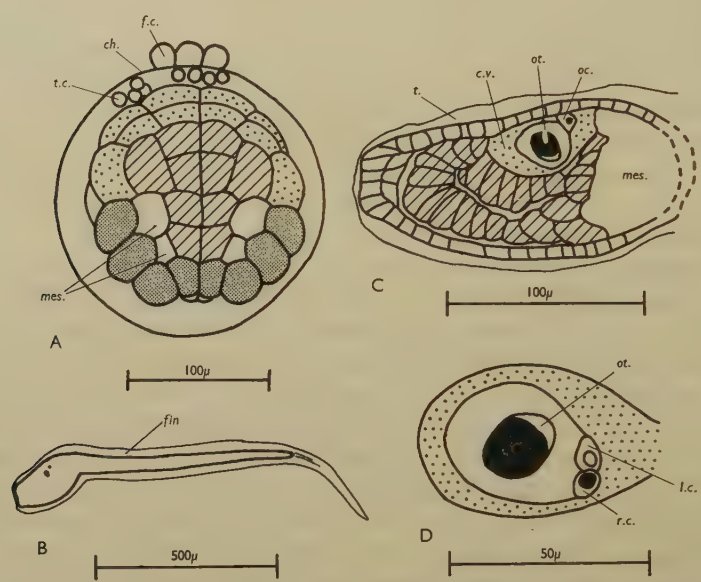

Fig. 1. A, Vegetal view of an embryo at the onset of gastrulation, most of the follicle cells and test cells omitted; B, larva; C, near-sagittal section of larval trunk; D, dorsal view of cerebral vesicle (whole mount). *Cross-hatched*: dark grey endoderm cells; *sparse stipple*: light grey crescent notochord and nerve cells; *close stipple*: yellow crescent muscle cells. *c.v.*, cerebral vesicle; *ch.*, chorion; *f.c.*, follicle cell; *l.c.*, lens cell; *mes.*, mesenchyme; *oc.*, ocellus; *ot.*, otolith; *r.c.*, retinal cell; *t.*, test; *t.c.*, test cell.

Development proceeds rapidly: the maturation divisions and first cleavage occupy the first hour after fertilization; early cleavages follow at half-hourly intervals; the embryo is gastrulating after 5 h and hatches within 14 h, at 21° C. The tail is flexed laterally to encircle the trunk of the embryo. A hatching enzyme is secreted which will digest the chorions of adjacent unfertilized eggs.

A recently hatched larva is shown in Fig. 1 B. The caudal fin runs above and

below the tail and projects behind it. On the trunk, a continuation of the ventral fin reaches the ventral papilla; the dorsal fin bifurcates, sending an arm to each of the two dorsal papillae. The muscle cells of the tail retain the yellow pigment. The cerebral vesicle (Fig. 1 C, D) contains a large otolith and a relatively small ocellus. The otolith is a single cell distended by a mass of black pigment 15 μ in diameter. It is difficult to discern the structure of the ocellus from longitudinal sections: Fig. 1 C shows the single retinal cell with its pigmented sphere (4 μ diameter). A single lens cell, containing a refractive body, may also be seen in fortunately orientated sections or dorsal views of whole mounts (Fig. 1 D). The lens cell lies to the right of the retinal cell, projecting very little above or in front of it.

The development of *S. mammiculata* appears to be as typical of its genus as is the adult form. Such features as the coloration of the egg and test cells, and general larval structure, are common also to *S. partita* (see Conklin, 1905) and to *S. plicata* (personal observation at Naples). Self-sterility and the hatching enzyme are found in these three species and the majority of other solitary ascidians. The occurrence of a visibly coloured mesodermal crescent may suggest an affinity between the Styelinae and Pyuridae (Millar, 1951, 1954). In addition to the instances given by Millar, a total of four species of *Styela* are known to form yellow crescents—the three cited above and *S. barnharti* Ritter & Forsyth (Berg & Humphreys, 1960). The only enterogonid ascidian known to do so is *Ascidiella scabra* (see Fautrez, 1940).

The phylogenetic value of the larval ocellus has been admirably demonstrated by Berrill (1948, 1950), but its structure in *Styela* required confirmation. Grave (1944), after a histological technique that resolved the cellular nature of only the muscles and notochord, recognized a pigmented cell as the ocellus of *S. partita* but homologized it to one of the three lens cells of enterogonid and pyurid larvae. Since the larva is light-sensitive, Grave was compelled to conclude that other unpigmented cells form a retina. Berrill (1948) described the ocellus of *S. yakatutensis* Ritter as possessing a single lens cell and a small pigmented retina (i.e. a reduced ocellus of the two typical cellular components, as described above). His later statement that 'the ocellus is reduced to a single pigmented lens cell in *Styela* species' (Berrill, 1950, p. 45) is at variance to his own results, the present description and optic theory. A lens transmits and refracts light: it would not be expected to carry an opaque pigment mass. A retina is commonly pigmented, to absorb light in the ascidian ocellus, or partly surrounded by a shield of pigment as in the vertebrate eye and possibly the botryllid photolith. Whether or not a lens cell is present in *S. partita*, Grave (1944) misconstrued the pigment cell which can only be the retina. The contention (Berrill, 1948) that all ascidian ocelli are homologous is supported here, for the small ocellus of *S. mammiculata* could easily be derived from that of the Pyuridae and Enterogona by a reduction of the number of both lens and retinal cells.

I gratefully acknowledge the facilities provided by the Director and Staff of the Plymouth Marine Biological Laboratory, and the receipt of a research grant from The Queen's University of Belfast.

SUMMARY

Styela mammiculata breeds during the summer, probably in August and September. Some eggs are self-sterile. Embryos show a yellow crescent of presumptive muscle cells. The larvae hatch by means of an enzyme. The larval sensory apparatus includes a large unicellular otolith and a small ocellus, which is composed of a single pigmented retinal cell and a single lens cell.

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REPRODUCTION, GROWTH AND MORTALITY OF *VENUS STRIATULA* (DA COSTA) IN KAMES BAY, MILLPORT

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(Text figs. 1–7)

The population of *Venus striatula*, on which this study is based, occurred on a bottom of fine clean sand at depths from 3–4 m to about 20 m. Kames Bay faces south, and is exposed to the prevailing winds which are from the south-west (Barnes, 1955). Wave action is often moderate and sometimes strong, and probably for this reason *V. striatula* does not occur here intertidally, although elsewhere it may extend above L.W.S.T. The fauna of the sublittoral region of the bay has been described by Clarke & Milne (1955) from two surveys, in 1938–39 and 1949. Since these surveys there has been a considerable increase in the numbers of *V. striatula*. Probably associated with this increase is a change in the carnivorous gastropod fauna, which has been discussed elsewhere (Ansell, 1960). Apart from these changes, the animal community in Kames Bay is still adequately described by Clarke & Milne's paper, and represents a typical '*Venus gallina* (= *V. striatula*) community' (Thorson, 1957).

The increase in the density of *V. striatula* is the result of a particularly successful settlement in 1955, estimated to represent an increase of a hundred-fold over the previous population. Hence, size distribution shows a single peak formed by animals derived from the 1955 settlement, and the population can be regarded in practice as composed of a single year-group. It was thus easy to study the rate of growth of the species in this area by sampling, and to demonstrate that certain markings on the shell are formed annually and can therefore be used to measure the rate of growth.

Some observations were also made on samples of animals from Hunterston Sands, Ayrshire, where the population consisted of animals of a number of different year-groups.

METHODS

The population was sampled regularly over the period 1956–59 by means of an anchor dredge (Forster, 1953). The gonads were fixed in Bouin's fluid in sea water, and embedded in ester wax (Steedman, 1947). Sections were cut at 10–12 μ and stained with Ehrlich's haematoxylin and eosin. Alternatively,

the macroscopic appearance of the gonads and smear preparations were used in assessing the state of development. In conjunction with observations of spawning on animals brought into the laboratory, this method gives, for *V. striatula*, an accurate estimate of the time and duration of spawning during the year. Daily sea-water temperature records were available for the period of the investigation from Keppel Pier, Millport, and these are given in Fig. 2.

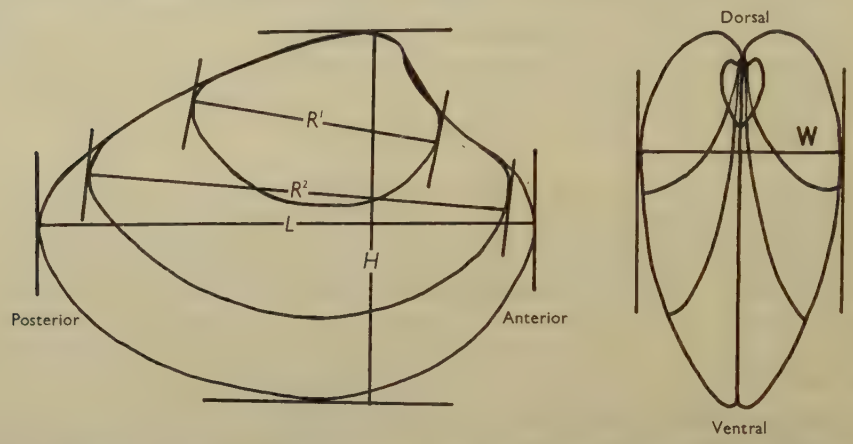

Fig. 1. Diagram to show the measurements made on the shells of adult *Venus striatula*. *H*, height; *L*, length; R^1, ring 1; R^2, ring 2; *W*, width, or thickness, or breadth.

Length was used as the measure of growth: height (dorso-ventral measurement) and thickness, or breadth (greatest lateral measurement) (Fig. 1) were used only to establish their relationships with length. Where ring number is referred to rather than age, age may be determined by adding one half to the ring number, since the true first ring, formed a few months after settlement, is rarely noticeable and is not included in the ring counts. Shells were measured to the nearest 0·1 mm.

Numerous animals were sexed prior to measurement, but in no case was any significant difference in shell proportions or in size found between males and females (cf. Fraser & Smith, 1928; Weymouth, McMillan & Rich, 1931; Quayle, 1952*b*).

THE REPRODUCTIVE CYCLE

The period just before spawning commenced forms a convenient point to begin a description of the annual cycle. During 1957 this condition was reached in late April to early May, when the temperature recorded at Keppel Pier was about 9° C. Ripe females were characterized at this stage by extended follicles containing large ripe ova. The follicles were closely packed, with little connective tissue between them. The ovaries formed a creamy white area in the visceral mass and extended dorsally and anteriorly over the digestive diverticula. In the fully ripe ovary the ovocytes were detached from

the follicle wall and occupied the centre of the lumen (Fig. 2, development stage 2).

The first animals showing signs of spawning were collected on 20 May. Before this, however, numerous animals had spawned on being brought into the laboratory, indicating that the bulk of the population was ripe. In 1958 spawning took place a little later than in 1957.

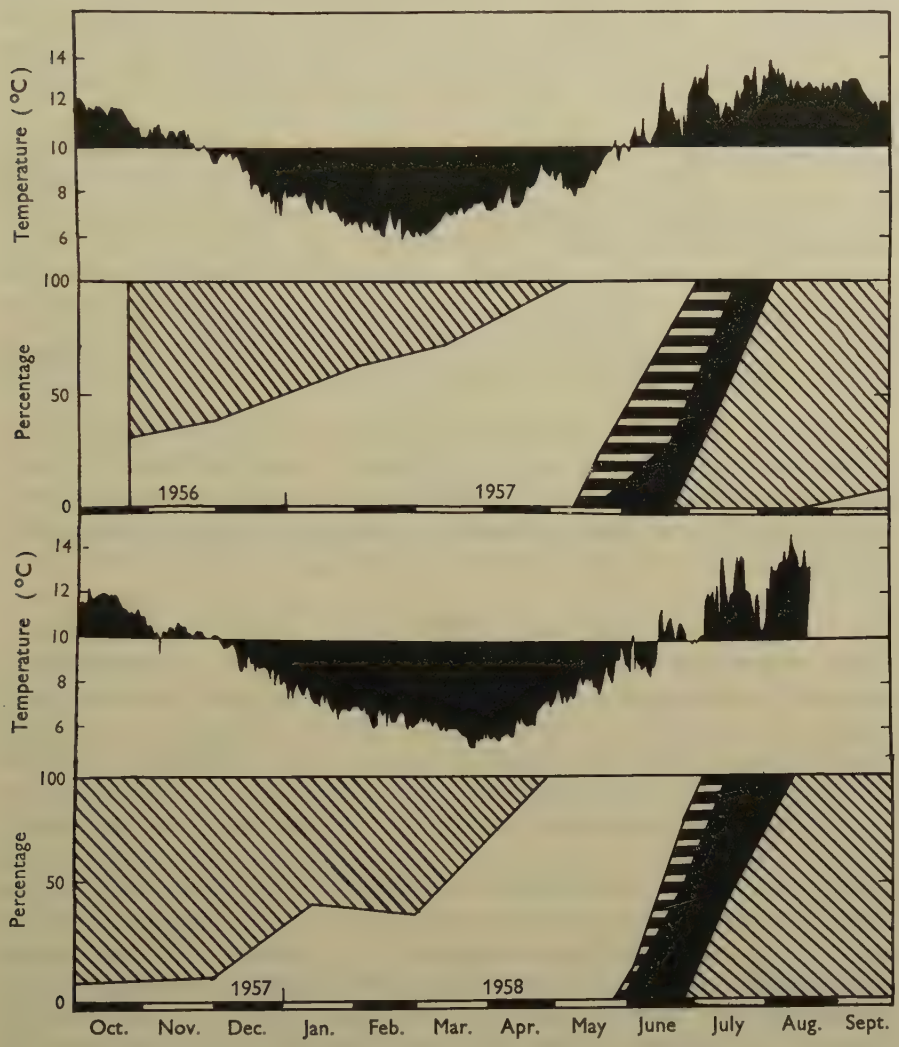

Fig. 2. *Venus striatula.* Diagram to show the percentage of females in each stage of the gonad cycle during the period October 1956–September 1958. Daily sea-water temperatures are also shown. Cross-hatched, development stage 1; unshaded, development stage 2; horizontally shaded, spawning?; shaded, partially spawned.

The female

In the female, spawning takes place at intervals during the season and is never complete, some residual ovocytes remaining in the ovary at the end of the spawning season. Similar residual ovocytes have been recorded in *Venerupis pullastra* (by Quayle, 1952 *a*), *Venus mercenaria* (by Loosanoff, 1937), *Cyprina islandica* (by Loosanoff, 1953) and in several other bivalves. In *Venus striatula*, as in *Venerupis pullastra*, these residual ovocytes persist throughout the winter and spring without being resorbed. Their ultimate fate is unknown, but it is probable that they are spawned with the ovocytes of the following year.

The development of young ovocytes in the ovary begins before spawning ends, so that the ovary passes directly from the spawning condition to an early stage of development. Immediately following spawning the follicles may sometimes become filled with vacuolated cells but the follicle walls do not collapse. Development at this stage is rapid and by September some of the animals may have passed into the second stage of development with the follicles filled with relatively large ovocytes. At this time, however, the ovary is still fairly small and is restricted to the ventral regions of the visceral mass.

Ovogenesis continues throughout the winter, although at a reduced rate. In late February and early March development is accelerated and the gonad extends dorsally and anteriorly. The ovocytes continue to increase in size and assume a pear-shaped form with a thin, stalk-like attachment to the follicle wall. By the end of April the ovaries are ripe.

The male

The cycle in the male is similar to that in the female. In the testis immediately before spawning the follicles are closely packed and are filled almost exclusively with ripe spermatozoa. These are typically arranged with the heads together forming dense bands within the follicles, an appearance which is particularly noticeable in sections, but also in some cases in smear preparations. In spawning animals the spermatozoa lie free in the follicles. Spawning apparently takes place at intervals; animals opened after spawning for some time in the laboratory still contained large numbers of spermatozoa. In some cases the gonads of such individuals were difficult to distinguish from those of fully ripe animals which had not spawned. When spawning is completed a number of spermatozoa are left in the gonad and ripe spermatozoa may be found at any time of the year. Immediately after spawning there is a development of vacuolated cells in the follicles although the presence of these may be masked by the spermatozoa which remain. Spermatogenesis begins rapidly and by early September the gonads have reached a considerable degree of development with spermatogonia and spermatocytes lining the walls of the

follicles and spermatozoa filling the lumen. Spermatogenesis continues throughout the winter, and the early months of the year are marked by an increase in the volume of the gonad. The animals are ready to spawn in late April.

Annual cycle

The annual reproductive cycle therefore consists of a brief period of recuperation following spawning followed by rapid development of the gametocytes. This development may overlap the spawning phase especially in the female. Development of the gonad is continuous until maturity in early May. This cycle is similar to that found in *Venerupis pullastra*[1] and to that of many North American species including *Venus mercenaria* (see Loosanoff, 1937) and *Paphia staminea* (see Quayle, 1943). It differs considerably from the cycle in many oysters where a period of fattening follows spawning, and where development of the gonad does not start until the following spring.

Sex ratio

Of the 765 animals examined in the course of the study of the breeding cycle, 388 were females and 377 males. An examination of a large sample of adults from Hunterston Sands indicated that the sex ratio remains the same at all ages. No hermaphrodite adults were found.

SPAWNING

Numerous observations have been made on the spawning of various members of the Veneridae. When the animals are ripe, introduction into the laboratory often causes a large percentage to spawn, the stimulus probably being rise of temperature, but possibly also mechanical disturbance.

Animals were brought into the laboratory and placed in filtered sea water in flat dishes of about 1 l. capacity. Forty or fifty animals were placed in each dish and examined at short intervals. The time after the start of observations at which each animal started to spawn was recorded and all spawning animals were removed to separate dishes. The rise of temperature to which the animals were subjected varied according to the season, but was in general about 5° C in summer and up to 10° C in winter.

The sexual products are first extruded into the suprabranchial chamber and are then discharged through the exhalant siphon, being carried by the exhalant stream for some distance. In male *Venus striatula* the sperm may reach a distance of 6–8 cm from the siphon. The distance varies in individuals and depends on the size of the animal and on the strength of the exhalant current. Shell movements play no part in the act of spawning in any of the Veneridae examined, an observation which agrees with that of Loosanoff (1937) on *V. mercenaria* and of Quayle (1952*a*) on *Venerupis pullastra*.

[1] Quayle, D. B., Ph.D. Thesis, University of Glasgow, 1948.

Spawning in both sexes is repeated at intervals throughout the spawning season, but no precise information on the length of the refractory period has been obtained. Quayle[1] found that the refractory period in *V. pullastra* was 2–3 days in both sexes. The intensity of spawning builds up to a peak in the middle of the spawning period and then drops. In Fig. 3 the intensity of spawning in groups of animals brought into the laboratory on different dates is indicated by plotting the number starting to spawn in each half hour period from the start of observations.

Early in the season the percentage number of animals which were induced to spawn in each group was low, and the time intervals between individual animals beginning to spawn, fairly long. Later in the season, as more animals in the population became physiologically ripe, the percentage spawning rose, and the time intervals became shorter. Animals in the dishes were exposed to stimulation by sexual products after the first animal had spawned, although each animal was removed as it was seen spawning. Under these conditions 'epidemic spawning' (Thorson, 1946) occurred, that is, a large number of the animals present were stimulated to spawn in a short time. In many cases over 50% of the animals present spawned within an hour of the first. Records of typical observations are shown in Fig. 3. Epidemic spawning is the result of continued mutual stimulation between the sexes and may, in *Venus striatula*, be initiated by either males or females. In cases where the first animal spawning was definitely seen, thirteen were started by males and eleven by females.

The time which elapsed between bringing the animals into the laboratory (i.e. the stimulus of rise of temperature) and the first spawning varied considerably, but was usually between 1·5 and 2 h (Fig. 3). During this period some physiological change must occur in the animals which eventually results in spawning. The nature of this change is unknown. In the female an important change in the eggs takes place between stimulation and the onset of spawning. In the ovary the eggs possess an intact germinal vesicle. Before spawning this breaks down and the first maturation division begins. The eggs are spawned at this stage. If the eggs are removed artificially from the ovary they do not mature, and artificial fertilization is therefore impossible.

No animals were found to spawn at temperatures lower than 11° C. Observations on the reproductive cycle recorded earlier suggest that in nature spawning may take place before the temperature reaches this point; some animals appeared to have spawned when the sea-water temperature recorded at Keppel Pier was still 9·0° C.

Other members of the Veneridae were observed to spawn in the following months: *V. ovata*, May; *V. casina*, May; *V. fasciata*, February, March, April, July; *Venerupis pullastra*, June, July; *V. decussata*, July; *Gafrarium minimum*, June.

[1] Quayle, D. B., Ph.D. Thesis, University of Glasgow, 1948.

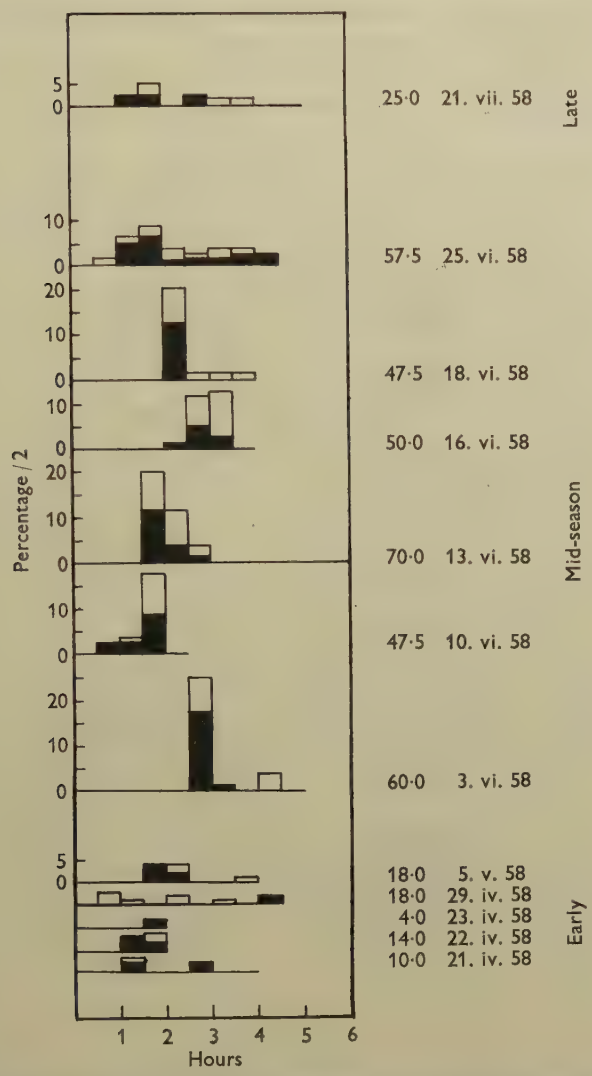

Fig. 3. *Venus striatula.* Diagram to show the intensity of spawning throughout the season. The histograms show the number of animals starting to spawn in each half hour period from the start of the observations. Shaded, males; unshaded, females.

DEVELOPMENT OF THE LARVA IN LABORATORY CULTURES

Eggs and sperm relatively free from detritus and other contamination were obtained from animals spawning in the laboratory. Fertilizations were made by adding a little sperm suspension to the egg suspension in a 500 ml. or 1 l. beaker, stirring continuously to avoid polyspermy and to insure fertilization

of all the eggs. The water was changed several times after fertilization to remove unfertilized eggs and excess sperm.

The fertilized eggs were kept mainly in 2 l. vessels containing water collected from Keppel Pier and filtered through Whatman no. 3 filter-paper.

Fig. 4. *Venus striatula*. Photomicrographs to show the shape and sculpture of the larval shell. Larva, 85 μ; larva, 90 μ; larva, 175 μ; larva, 185 μ; larva, 195 μ; post-larva, 300 μ; post-arva, 640 μ.

For some fertilizations sea water filtered through a Berkfeld bacterial filter was used in an attempt to restrict bacterial growth. The water in the vessels was changed at intervals of a few days.

The egg is surrounded by a gelatinous membrane within which cleavage

takes place. Cleavage is of the typical bivalve type and the trochophore larva is formed within 24 h of fertilization. At this stage the gelatinous membrane is shed and the larva becomes free-swimming. The secretion of the larval shell by the shell gland during the second day following fertilization brings the larva into the straight-hinge veliger stage. Up to this point the source of nourishment for the developing larva has been the yolk stored in the egg, but on the third to fourth day following fertilization the gut is fully formed and the larva begins to feed on nanoplankton.

When the larva reached this stage in laboratory cultures they were fed daily by the addition of a few ml. of a culture of *Monochrysis lutheri* Droop. When larvae were observed crawling, a small dish of sand was placed in the vessel. Larvae settled in the sand, but some settlement also took place on the glass bottom of the culture vessel. In cultures to which no sand was added the larvae settled normally on the bottom of the vessel.

Larvae attributed to *Venus striatula* have been described by Jørgensen (1946) from the Øresund, and by Rees (1950, 1951) from the North Sea. The identification of the larva by Rees was tentative, but is confirmed by observations made here.

Useful characters for the identification of the larva of *V. striatula* are the shape and sculpture of the larval shell. Photomicrographs were taken to illustrate these in larvae of different sizes (Fig. 4). Prodissoconch I is immediately distinguishable from the later-formed Prodissoconch II. The former bears small, punctate markings and is 90–110 μ long. Prodissoconch II bears concentric lines of varying degrees of clarity, but most are well marked. Especially towards the ventral edge, the shell may be of a light, brownish orange colour.

Setting occurs when the larva has reached a length of 200–220 μ, although some larvae may set when only 190 μ long. A few remain planktonic until 280 μ long. Jørgensen (1946) found young bottom stages with Prodissoconch II, 210–225 μ long and a single one with Prodissoconch II, 280 μ long.

EFFECT OF TEMPERATURE ON THE RATE OF GROWTH OF THE LARVA

Fertilizations were made as described earlier, and the larvae allowed to develop at room temperature (about 15·0° C) for 2 days, that is, until the veliger stage was reached and the larval shell had grown to a length of 100–120 μ. The larvae were then divided at random between the eight 600 ml. beakers which were used during the experiment, to give a density of about 10 per ml. in each vessel. Throughout the experiment, the sea water present in the vessels was removed every second day through a stainless steel mesh filter, and fresh sea water added. The sea water used was collected from Keppel Pier Millport, and filtered through Whatman no. 3 filter-paper to remove the larger

planktonic organisms and debris. Aliquots of a culture of *Monochrysis lutheri* Droop were added to the cultures each day to maintain a concentration of approximately 500 cells per ml. of water.

The results of the two experiments carried out are summarized in Tables 2 and 3. On the sixteenth day following fertilization, the fastest growing larvae in the cultures were beginning to set and the experiments were concluded at this point.

The larvae in cultures kept at temperatures between 10·0° and 22·5° C inclusive behaved normally. They swam vigorously and remained in suspension in the sea water without mechanical agitation of the cultures. Cells of *Monochrysis* could be seen being moved by the cilia and crystalline style in the stomach, while the digestive diverticula were coloured green by the ingested food particles.

Larvae kept at 5·0° C in Expt. 1 failed to grow. These larvae were sluggish, and transparent, with little or no food in the gut. Some of the larvae at this temperature were alive at the end of the experiment, 16 days after fertilization, but mortality was estimated as at least 85%. A similar mortality occurred in the cultures kept at 26·0° C, although in these food was observed in the stomachs of the larvae. In spite of this, little growth occurred, and it is probable that this temperature is near the upper limit of tolerance for these larvae.

Mortality in cultures kept at temperatures between 10·0° and 22·5° C was low. In those kept at 12·0, 14·0, and 16·0° C mortality was estimated at the conclusion of the experiments as not more than 10%. At 10·0° C and at 22·5° C mortality was higher, an observation which agrees with that of Loosanoff, Miller & Smith (1951) who found that mortality was greatest in those cultures of *Venus mercenaria* in which the growth of the larvae was slowest. It is to be expected that mortality in cultures will be greatest at temperatures closest to the tolerance limits of the species.

Only the fastest growing larvae in the cultures reached metamorphosis before the experiments were concluded, so that no information is available on the size at metamorphosis at the different temperatures. Loosanoff *et al.* (1951) found no indication that larvae of *V. mercenaria* grown at lower temperatures were reaching a larger size before setting than those grown at higher temperatures.

The two experiments showed agreement in general, although there are some discrepancies. In particular, larvae in the culture kept at 16·0° C in the first experiment grew considerably faster than those in the similar culture in the second experiment, and in fact grew faster than the larvae kept at 20·0° C. The results indicate, however, that the rate of growth of the larva increased with increase in temperature in the range 5·0°–16·0° C. Growth at 20·0° C is similar to that at 16·0° C, and it seems likely therefore that the maximum mean rate of growth might occur at about 18·0° C. At temperatures above

20·0° C growth of the larvae is retarded. Significant amounts of growth were obtained in the range 12·0°–22·5° C.

The range of variation in size of the larvae was different in different cultures. In Table 3 are given the maximum and minimum lengths of larvae at each temperature for each day on which measurements were taken, the table being based on the combined results of the two experiments. Representative examples of the length/frequency distribution of the larvae are given in Fig. 5.

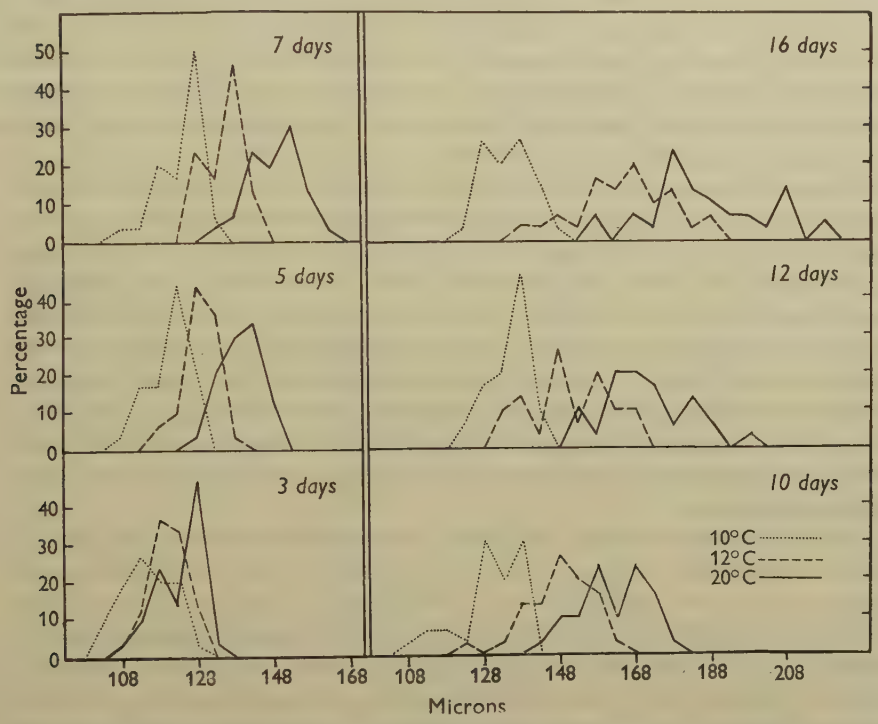

Fig. 5. *Venus striatula*. Length/frequency distributions (expressed in percentages) of larvae of different ages, grown at temperatures of 10·0, 12·0, and 20·0° C, in Expt. 2.

The range of variation in size tends to increase as the larvae grow older, especially in cultures kept at higher temperatures. The greatest individual rate of growth occurred in the 20·0° C culture, but this was offset by the wide variation which occurred.

The two experiments described were carried out on the shelled larva of *V. striatula*. Earlier, experiments were attempted in which newly fertilized eggs were transferred to different temperatures and their subsequent development followed. Although incomplete, these observations are included here since they indicate that the temperature tolerance of the earlier stages of development may be different from that of the veliger larva.

Eggs fertilized at room temperature (about 15·0° C) and then transferred with gradual rise of temperature to 26·0° C failed to develop normally. Segmentation was abnormal, small pieces of the egg became completely detached, and in some a bubbling appearance of the cytoplasm developed. The eggs soon died. At 22·5° C the same processes occurred.

At the other end of the temperature scale, at 5·0° C, the eggs were apparently not injured and division occurred, although at a much reduced rate. Most of the larvae produced were abnormal, and mortality was almost 100% before the veliger stage was reached. Between 10·0° and 18·0° C development was normal, but the time between the first and second, and the second and subsequent divisions, was greater at lower temperatures. At 20·0° C there was some interference with the normal course of early development, and a large proportion of the larvae produced were abnormal.

These observations on the early development of *V. striatula* at extreme temperatures are in agreement with results found for other bivalves. Seno, Hori & Kusakabe (1926) found that at 16·0° C few of the eggs of *Ostrea gigas* would develop to the shelled larva and that the larvae which were produced were abnormal and soon died. At 14·0° C segmentation was so abnormal that no shelled larvae were produced. In *Venus mercenaria*, Loosanoff *et al.* (1951) found that eggs placed in water at 15·0° C within 3 h of fertilization would not develop normally. Some would develop into trochophore larvae, usually of abnormal appearance. At 33·0° C an abnormal development and heavy mortality occurred if the fertilized eggs were immediately transferred to such a high temperature.

Measurements were made of a large number of larvae of different sizes. Those of the larvae grown at 16·0° C in the two experiments, together with some of those larvae grown at 20·0° C, are given in Table 4 with the relationship between length and height of the larvae from the straight-hinge stage to metamorphosis. The relationship is simple and direct with no radical change in shape with age. Dispersion is slight, and is partly due to differences in the position in which the larvae lie on the slide; especially in larger larvae the curved shell causes them to lie obliquely. The size of the smallest larva shown was $108 \times 83\,\mu$, the largest $235 \times 210\,\mu$.

THE RATE OF GROWTH OF THE ADULT

Results from sampling (1955 *settlement*)

The results obtained from repeated sampling over the period October 1956 to December 1958 are summarized in Table 5 as mean size of the dominant year group and the standard deviation. These results do not include information on growth during the first year following settlement, but samples of spat collected during 1956, 1957 and 1958 indicate that after settlement in the summer months the spat grow to a length of 0·1–0·3 mm before their first

winter, when growth slows, or ceases completely. The main year-group dealt with here is therefore that of the settlement of 1955, which in October 1956 was between 1 year 2 months and 1 year 5 months old.

The results show clearly that noticeable growth is restricted to the summer months of May, June, July and August. In Table 6 and Fig. 7 the mean increment/day added by the animals between each sampling date during 1957 is shown. Growth is slow during May, increases during June and is maximal during July. Thereafter the rate is reduced.

The shell of *V. striatula* bears a number of more or less well defined rings on the surface. The series of samples taken shows clearly the annual nature of these rings, and their usefulness as an indication of age. The measurements of the rings in the samples are summarized in Tables 7 and 8, and in Fig. 6. In 1957, the first sign of renewal of active growth after the winter months occurred in the collection taken on 16 May. In this collection some animals showed a small edge of obviously new growth marked off from the previously formed shell by a ring. By 7 June, all animals in the collection were showing a similar edge of newly formed shell, and a ring was distinguishable in each. The mean length of this ring in these and subsequent samples corresponds to the mean length of the animals in the collections taken during the winter of 1956–57. A similar ring, corresponding in length to the length of the animals during the winter of 1957–58 became apparent when growth restarted in 1958. No rings were formed on the shell between these two dates. One ring only therefore is formed each year, which becomes apparent at the recommencement of growth after the winter, and such rings may be used for the determination of the age and rate of growth of individual animals.

Results from ring measurements

To determine the form of the general growth curve of the species, measurements of the annual rings on the shells of older individuals from Kames Bay have been used. As the animal ages the annual increment of growth in length lessens, so that the later-formed rings are closely packed. For this reason it is usually impossible to distinguish the later-formed rings in old shells. However, shells have been found in which ten rings were distinguishable, so that the duration of life may be ten or eleven years and probably more in some cases.

In all, 372 measurements of annual rings in older shells from Kames Bay have been made. These are grouped according to ring number in Table 9. Since the measurements for each ring include those of a number of different years, the means show a trend of growth unaffected by annual variations in rate caused by short term environmental fluctuations. Comparison of these values with those derived from measurements of the rings of animals from the 1955 settlement, and with measurements of the total length of animals from the 1955 settlement taken during the winter months, indicate that the 1955

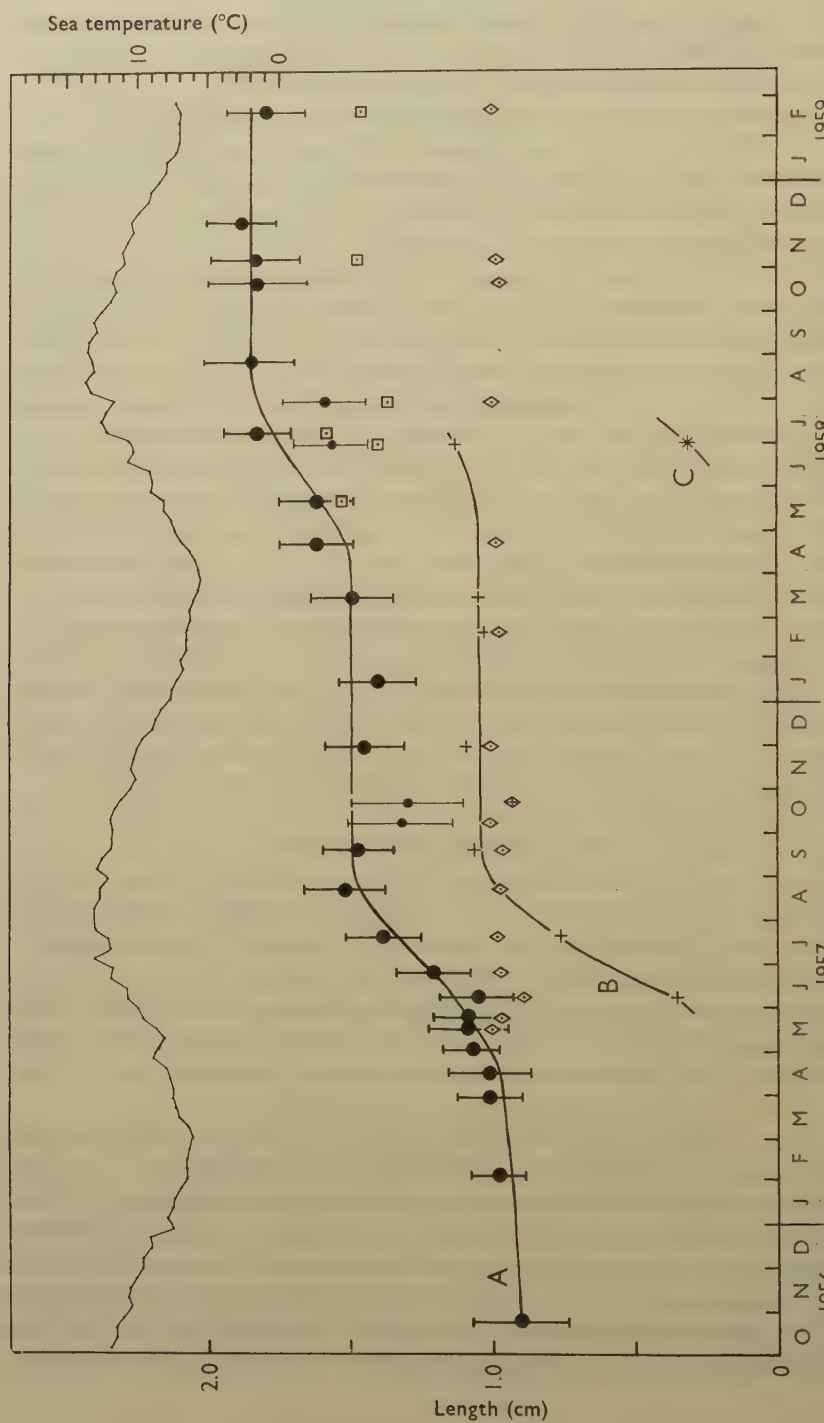

Fig. 6. Growth of young *Venus striatula* in Kames Bay, Millport. A, 1955 settlement; B, 1956 settlement; C, 1957 settlement. The mean length of ring 1 (diamonds) and ring 2 (squares) for the 1955 settlement are also shown.

settlement showed a higher mean rate of growth than that of the earlier settlements, in spite of the greater population density.

SHELL PROPORTIONS IN THE ADULT STAGE

Table 10 shows the height/length and thickness/length relationships for animals between 0·75 and 2·25 cm in length. The ratios remain relatively constant throughout adult life, except in very large individuals where growth tends to become completely transverse, with no corresponding increase in length.

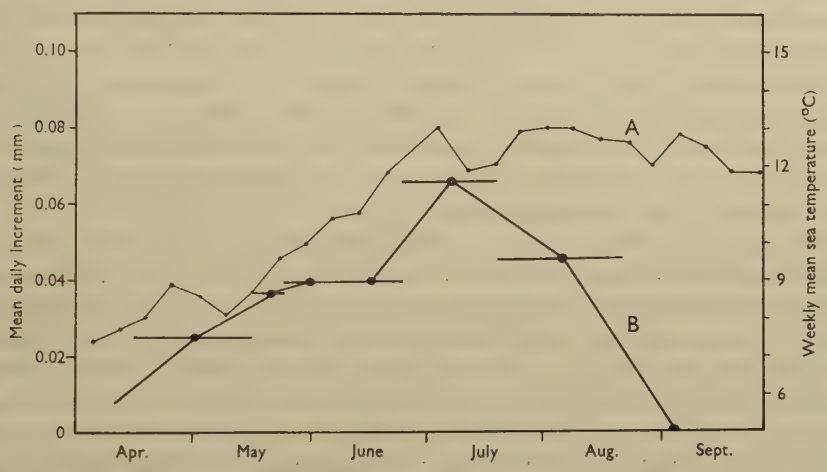

Fig. 7. *Venus striatula.* Daily increments in length of the 1955 settlement in Kames Bay, during 1957. The weekly mean sea-water temperature for the period is also shown.

MORTALITY

A rough estimate of mortality was obtained from counts of the number of dead shells present in samples compared with the number of living animals. Only one cause of mortality was definitely established—that of predation by the gastropod drill *Natica alderi* Forbes. Approximately 15% of the population in the first, 5% in the second, and 1–2% in the third year of life of the bivalves were bored by *Natica* (Ansell, 1960). Birkett & Wood (1959) found that young *Mactra stultorum* from the Dogger Bank often bear up to five bore holes made by *Natica poliana*. In *Venus*, no evidence was found of such 'wasteful killing': multiple perforations were relatively rare and never was more than one *complete* perforation found in a shell.

Apart from the mortality due to the activities of *Natica alderi*, there was a considerable 'residual mortality', the causes of which are unknown. In the period from the time when the bivalves reached a length of 2–3 mm until October 1956 (approximately the first year of life) this residual mortality

accounted for the death of about 25% of the population. The total mortality during this period was therefore about 40%. During the second year, the total mortality was about 33% of the animals remaining alive at the end of the first year. During the third year the mortality was approximately the same.

It was thought that the mortality might in some way be the result of the high density of the 1955 settlement. A series of experiments was therefore carried out during 1957 in a large outdoor tank at Millport which had originally been used for the tank-breeding of oysters. Wooden boxes, approximately 0·1 m^2 in area and 22 cm in depth were filled with sand to a depth of about 10 cm, and placed on the bottom of the tank, which was then filled with sea water to a depth of about 3 ft. The sand was obtained from Kames Bay, and sieved before use.

Venus striatula from Kames Bay were placed in the boxes to give artificial population densities of 10, 30, 100, 200, 500 and 1000 per one-tenth of a square metre. These populations were set up on 31 March 1957, and the experiments were brought to a close on 28 June 1957, when all the animals both alive and dead were preserved.

The results obtained are summarized in Table 11. Over the range of population density from 300/m^2 to 10,000/m^2 the percentage mortality during the 3 months of the experiment was apparently not affected by population density in any way. The growth increments given in the table are too small to show whether there is any significant density affect on growth, but are similar to those which occurred in members of the natural population over the same period.

In a similar experiment with *Venerupis semidecussata*, Ohba (1956) found that percentage mortality increased with population density and that there was a linear relationship between the two in populations of less than 5000/m^2. Moreover, individual growth was less in the denser populations. However, the mortality recorded in his experiments was much higher than that recorded here (68·8% in a population of 10,000/m^2), so that the conditions were apparently more stringent.

DISCUSSION

It is difficult to draw conclusions on the effect of environmental conditions on the rate of growth, but some general observations may be made. During the early part of the season growth follows temperature closely, but drops in August although the temperature is then maximal. This relation is similar to that in the Pismo clam, *Tivela stultorum* (see Weymouth, 1923; Coe, 1947), and in *Venerupis pullastra* (see Quayle, 1952*b*). Both Weymouth and Quayle consider that the falling off of growth at a time when the water temperature is still high, is connected with breeding, a view advanced earlier by Orton (1928), who found that growth of the shell of *Ostrea edulis* took place in the spring and autumn, but not in the summer, when the animals were breeding. Orton

suggested that there is a physiological antagonism between reproductive activities and shell growth. In *Venus striatula* the maximum rate of growth occurs during the same months as spawning, when the proliferation of the gonad and the growth of young ovocytes is at a minimum. Reduction in the rate of growth in August occurs at a time when spawning is near completion and renewed growth of young ovocytes is taking place. There is a physiological antagonism in this case between growth of the shell, and proliferation of the gonad and growth of young ovocytes, in contrast to *Ostrea* where the reduction in shell growth is apparently connected with the larviparous habit.

The same trend of growth also takes place in the two species of *Mytilus* and in other invertebrates found in California (Coe & Fox, 1944). These authors suggest that food supply is the factor which controls the growth rate rather than small changes in temperature. At Millport, the spring diatom increase (Marshall & Orr, 1927) coincides with proliferation of the gonad of *Venus striatula*, while growth does not start until about a month later, when it follows closely the increase in temperature of the sea water.

The ring measurements show that there is a considerable variation in the rate of growth. This is partly the result of annual variation in the population as a whole; but even between animals of the same year-group there is still much variation. This may be ascribed to three sources: first, there is a period of approximately 3 months in the summer during which settlement may take place, so that animals of the same year-group may differ in age by as much as 3 months; secondly, the rate of growth of individuals may be affected by local differences in the environment, although in the small area of Kames Bay this factor is probably not important; and thirdly, the observed differences in the rate of growth may be the result of inherited or acquired physiological factors.

The development of the gonad of *V. striatula* appears to depend on the availability of food material from the environment rather than on food reserves stored within the animal. In 1958, the sea-water temperature as recorded at Keppel Pier was lowest towards the end of March, about a month later than in 1957. In spite of this, the development of the gonads followed the same pattern in both years. The proliferation which takes place in the months of February and March coincides with the period when the phytoplankton in the water is increasing. At this time the digestive diverticula of the animals become deep green in distinction to the rather pallid appearance they have during November and December. It thus appears that the proliferation of the gonad takes place in response to increased food supply rather than to increased temperature, and presumably occurs whenever the amount of food obtained by the animal is sufficient to exceed the amount required by other metabolic processes (apart from growth of the shell). Under these circumstances, an increase in phytoplankton density, or an increase in temperature leading to an increased rate of water filtration by the bivalve, would be expected to accelerate the rate of proliferation of the gonad.

The observations made on the months during which spawning takes place in members of the Veneridae extend those of other workers. The observations for *Venerupis pullastra* agree with those of Quayle (1952 *a*), who observed this species spawning at Millport during May, June and July, and found larvae in the water during July, August and September. Lebour (1938) records that this species breeds at Plymouth in June and July, and also in September and October. In the North Sea, larvae which were probably of this species (Rees, 1950, 1951, 1954; ?*Paphia*) occurred in the plankton in September, October, November and December. In each area, therefore, this species breeds during the period when the sea temperature is at, or approaching, the annual maximum. The larvae of *Venus striatula* occur in the North Sea in September, October and November (Rees, 1951, 1954; Venerid B) when the sea temperature is maximal. Lebour states that this species breeds at Plymouth in February, March and May. Jørgensen (1946) has recorded larvae of this species from the Øresund from September 1941 to February 1942 and in August and September 1942. The larvae were most numerous in October and November at about the time when the water temperature was maximal. Those two species of the Veneridae for which most information on the breeding season is available therefore conform with the generalization of Orton (1920) that species nearer the lower limit of their geographical range of temperature should breed during the warmer months of the year.

The figures for mortality indicate that there is a fairly sharp decline in the population during the early years following settlement and that few of the animals live to complete the maximum life span possible. These figures apply only to those causes of mortality which leave the empty shell intact in the same position which it occupied in life. A possible cause of mortality which does not fulfil this condition is predation by bottom-feeding fish, many of which are known to take bivalves. However, fish are not abundant in Kames Bay and their effect on the population of *Venus striatula* is probably small.

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SUMMARY

Seasonal changes in the gonads of both sexes of *Venus striatula* were followed by macroscopic and microscopic examination, in over 700 animals from a population consisting mainly of one year-group. The sex ratio was approximately equal, and no hermaphrodite adults were found.

The time and duration of the spawning period of *V. striatula* during 1957 and 1958 were found. Spawning in both sexes takes place at intervals throughout the period. Intensity of spawning builds up to a peak near the middle of the season and then drops. In the laboratory no animals spawned at temperatures below 11·0° C. Spawning may be induced by rise of temperature or stimulation by sexual products. 'Epidemic spawning' may be initiated by either males or females. Natural spawning was observed in seven species of the Veneridae. Shell movements play no part in the spawning act. Artificial fertilization is impossible since eggs removed from the ovary still possess the germinal vesicle intact, and this is not broken down in sea water.

The veliger larva of *V. striatula* was cultured from eggs obtained from natural spawnings fertilized in the laboratory, and reared until settlement took place. Laboratory experiments were carried out to find the effect of temperature on larval development and growth. Larvae were grown in two experiments at temperatures between 5·0° and 26·0° C and observations on the development of the eggs were also made.

The rate of growth of young *V. striatula* was found from a series of samples taken over the period October 1956 to January 1959. The season of growth was found to be restricted to the summer months of May, June, July and August. Certain markings on the shell are shown to be of an annual nature, and a general growth curve for the species has been constructed from measurements of 372 of such rings. This follows the usual sigmoid form.

The rate of mortality among the 1955 spatfall in Kames Bay is described. In artificial populations, mortality was found to be independent of the population density.

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TABLE 1. *VENUS STRIATULA*. PERCENTAGE OF ANIMALS IN EACH STAGE OF THE GONAD CYCLE, 1956–58

	Females					Males					
Date	Dev. I	Dev. II	P.S.?	P.S.	Total ♀	Dev.	P.S.?	P.S.	Total ♂	Total	% ♀
23. x. 56	69	31	0	0	13	100	0	0	17	30	43·3
30. xi. 56	61	39	0	0	13	100	0	0	17	30	43·3
1. ii. 57	36	64	0	0	11	100	0	0	19	30	36·7
13. iii. 57	28	72	0	0	18	100	0	0	12	30	60·0
3. v. 57	0	100	0	0	24	100	0	0	15	39	61·5
9. v. 57	0	100	0	0	21	100	0	0	20	41	51·2
20. v. 57	0	77	23	0	17	76	16	4	24	41	41·5
22. vi. 57	0	18	48	30	22	40	32	24	12	34	64·7
19. vii. 57	63	0	0	37	19	40	33	27	15	34	55·9
21. viii. 57	100	0	0	0	15	100	0	0	24	39	38·5
26. ix. 57	92	8	0	0	25	100	0	0	30	55	45·5
30. xi. 57	88	12	0	0	25	100	0	0	34	59	42·4
14. i. 58	56	44	0	0	25	100	0	0	17	42	59·5
27. ii. 58	60	40	0	0	25	100	0	0	20	45	55·6
21. iv. 58	4	96	0	0	20	100	0	0	20	40	50·0
3. vi. 58	0	85	5	10	20	94	0	6	13	33	60·6
25. vi. 58	0	20	28	52	25	50	31	19	16	41	61·0
15. vii. 58	44	0	0	56	25	42	29	29	24	49	51·0
14. viii. 58	100	0	0	0	25	100	0	0	28	53	47·2

TABLE 2. *VENUS STRIATULA* LARVAE IN CULTURES

(Number measured (*N*), and mean length (*l*) in microns)

Days...	3		5		7		10		12		14		16	
Temp.	*N*	*l*	*N*	*l*	*N*	*l*	*N*	*l*	*N*	*l*	*N*	*l*	*N*	*l*
							Experiment 1							
5°	50	115·3	30	117·8	50	117·6	33	117·1	30	117·3	—	—	50	117·7
10°	30	113·4	50	116·3	50	118·8	30	119·2	30	123·6	30	127·7	30	123·5
12°	40	117·8	50	124·4	50	133·9	30	139·2	30	146·9	30	154·8	30	158·0
14°	50	121·8	50	132·0	40	138·5	30	148·9	30	159·2	30	162·2	30	164·7
16°	50	123·1	50	136·2	30	152·7	50	164·5	30	166·8	30	186·9	30	192·4
20°	50	122·6	30	140·5	30	148·8	30	152·8	30	162·4	30	174·3	30	183·7
22·5°	50	125·0	30	138·5	30	136·7	31	142·9	30	150·5	30	155·8	—	—
26°	50	117·2	30	121·2	30	118·9	—	—	30	122·5	—	—	—	—
							Experiment 2							
10°	30	115·7	30	122·2	30	125·6	30	130·5	30	135·8	—	—	30	135·3
12°	30	120·9	30	129·5	30	136·4	30	148·7	30	152·5	—	—	30	167·2
14°	30	121·3	30	132·8	30	146·6	30	156·0	30	157·8	—	—	30	177·9
16°	30	123·1	30	136·4	30	147·8	30	156·8	30	158·4	—	—	30	174·2
20°	30	124·0	30	140·7	30	150·0	30	163·3	30	172·2	—	—	30	189·3
22·5°	—	—	30	137·1	30	147·5	30	156·8	30	161·7	—	—	30	183·6
26°	—	—	30	123·9	30	123·1	30	124·4	—	—	—	—	—	—

TABLE 3. MAXIMUM AND MINIMUM LENGTHS, IN MICRONS, OF LARVAE OF *VENUS STRIATULA* IN CULTURES

(Based on two experiments)

	Temperature															
	5·0° C		10° C		12° C		14° C		16° C		20° C		22·5° C		26° C	
Days	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
3	125*	100*	129	100	129	102	131	110	130	108	132	108	131*	108*	128*	105*
5	125*	108*	130	103	136	105	140	121	145	120	152	120	151	118	130	110
7	126*	110*	135	108	145	120	150	125	165	135	170	121	160	120	130	110
10	125*	110*	140	108	161	125	170	131	185	130	181	120	175	125	135†	115†
12	125*	110*	145	110	170	132	178	135	190	145	200	121	190	121	130*	115*
16	—	—	148	110	190	135	200	153	220	160	230	140	210†	145†	—	—

* Based on Expt. 1 only. † Based on Expt. 2 only.

TABLE 4. LENGTH/HEIGHT RELATIONSHIP OF LARVAE OF *VENUS STRIATULA*

Mid point length (μ)	No. of specimens	Mean height (μ)	Height/length
108	4	088	0·815
113	4	094	0·832
118	17	094	0·797
123	30	098	0·797
128	41	103	0·805
133	14	111	0·835
138	46	113	0·819
143	25	119	0·832
148	40	123	0·831
153	44	128	0·837
158	43	132	0·835
163	22	137	0·840
168	49	145	0·863
173	38	149	0·861
178	46	155	0·871
183	35	159	0·869
188	27	166	0·883
193	15	169	0·876
198	26	176	0·889
203	11	180	0·887
208	21	185	0·889
213	7	188	0·883
218	10	195	0·894
223	2	198	0·888
228	2	206	0·903
233	1	208	0·893

TABLE 5. SUMMARY OF DATA FROM SAMPLING OF *VENUS STRIATULA*. 1955 SETTLEMENT, KAMES BAY

(Length, in tenths of a millimetre)

Date	*N*	Range	Mean	Standard deviation
23. x. 56	714	21–120	89·85	16·61
1. ii. 57	170	59–118	97·82	9·67
29. iii. 57	630	48–137	100·98	11·28
15. iv. 57	774	24–134	100·96	14·31
3. v. 57	350	77–137	107·54	9·72
16. v. 57	92	61–134	108·27	13·88
24. v. 57	281	46–142	108·28	11·95
7. vi. 57	121	53–128	105·60	12·97
24. vi. 57	409	70–160	120·70	13·12
19. vii. 57	335	89–175	138·12	13·15
21. viii. 57	207	108–185	151·55	14·47
8. ix. 57	518	114–186	147·18	12·32
7. x. 57	323	81–185	131·78	18·38
21. x. 57	405	71–198	129·27	19·89
30. xi. 57	440	113–189	144·64	13·95
14. i. 58	360	99–173	139·89	13·69
17. ii. 58	499	104–175	138·92	13·14
14. iii. 58	1151	106–191	148·77	14·51
21. iv. 58	614	123–200	161·50	13·25
21. v. 58	454	124–205	161·46	13·34
30. vi. 58	179	133–193	156·35	12·63
8. vii. 58	320	145–215	182·61	11·49
30. vii. 58	541	124–215	158·88	14·86
26. viii. 58	309	148–218	184·82	13·21
22. x. 58	138	135–220	182·54	14·81
7. xi. 58	212	144–235	183·02	15·80
3. xii. 58	66	158–224	188·10	12·49
18. ii. 59	215	151–222	179·40	13·88

TABLE 6. *VENUS STRIATULA*. DAILY INCREMENTS IN LENGTH OF 1955 SETTLEMENT DURING 1957, IN MM.

Period From	Period To	Days	A Mean length – Mean R1 start	B Mean length – Mean R1 end	B – A increment	Increment per day
15. iv. 57	16. v. 57	31	0	0·774	0·774	0·0250
16. v. 57	24. v. 57	8	0·774	1·067	0·293	0·0366
24. v. 57	7. vi. 57	14	1·067	1·623	0·556	0·0397
7. vi. 57	24. vi. 57	17	1·623	2·296	0·673	0·0396
24. vi. 57	19. vii. 57	25	2·296	3·945	1·649	0·0660
19. vii. 57	21. viii. 57	33	3·945	5·413	1·468	0·0445

TABLE 7. *VENUS STRIATULA*. 1955 SETTLEMENT, KAMES BAY

(Length of first growth ring, in tenths of a millimetre)

Date	*N*	Range	Mean length	Standard deviation
16. v. 57	91	61–124	100·53	12·48
24. v. 57	281	46–130	97·61	11·44
7. vi. 57	120	32–110	89·37	13·49
24. vi. 57	399	42–134	97·74	12·57
19. vii. 57	322	40–136	98·67	13·06
21. viii. 57	207	53–125	97·42	13·30
8. ix. 57	519	50–129	96·50	10·43
7. x. 57	223	59–128	101·09	9·79
21. x. 57	334	62–125	93·48	10·12
30. xi. 57	442	68–128	100·98	9·81
17. ii. 58	505	55–120	97·23	9·50
21. iv. 58	613	48–128	98·60	10·55
21. v. 58	455	43–125	98·20	10·03
30. vii. 58	539	55–120	100·16	9·69
7. xi. 58	212	66–125	98·86	3·87
18. ii. 59	215	68–126	100·02	8·77

TABLE 8. *VENUS STRIATULA*. 1955 SETTLEMENT, KAMES BAY

(Length of second growth ring, in tenths of a millimetre)

Date	*N*	Range	Mean length	Standard deviation
21. v. 58	454	116–194	152·30	13·16
30. vi. 58	180	115–178	140·50	12·77
8. vii. 58	324	124–192	158·66	12·27
30. vii. 58	541	101–185	136·96	12·90
7. xi. 58	212	114–190	147·03	8·01
18. ii. 59	215	119–176	146·72	11·10

TABLE 9. *VENUS STRIATULA*. RING MEASUREMENTS IN KAMES BAY

Mid Point	R 1	R 2	R 3
043	1	—	—
048	1	—	—
053	6	—	—
058	4	—	—
063	11	—	—
068	9	—	—
073	10	—	—
078	6	—	—
083	12	—	—
088	9	—	—
093	4	—	—
098	4	3	—
103	4	1	—
108	2	4	—
113	3	4	—
118	1	9	—
123	1	12	—
128	1	11	1
133	0	8	1
138	0	13	1
143	0	8	1
148	1	7	6
153	—	3	11
158	—	2	8
163	—	4	15
168	—	1	12
173	—	—	5
178	—	—	10
183	—	—	3
188	—	—	8
193	—	—	3
198	—	—	4
203	—	—	1
N	90	90	90
Mean (cm.)	0·78	1·32	1·68

Mid Point	R 4	R 5	R 6	R 7	R 8	R 9	R 10
163	1	—	—	—	—	—	—
168	1	—	—	—	—	—	—
173	2	—	—	—	—	—	—
178	7	—	—	—	—	—	—
183	8	—	—	—	—	—	—
188	6	1	—	—	—	—	—
193	11	1	—	—	—	—	—
198	2	3	1	—	—	—	—
203	5	4	0	—	—	—	—
208	6	5	0	—	—	—	—
213	3	2	0	—	—	—	—
218	1	2	2	—	—	—	—
223	2	1	3	—	—	—	—
228	—	0	2	—	—	—	—
233	—	2	3	—	—	—	—
238	—	1	0	1	—	—	—
243	—	1	0	1	—	—	—
248	—	—	1	2	—	—	—
253	—	—	0	0	1	—	—
258	—	—	1	0	—	—	—
263	—	—	1	0	—	—	—
268	—	—	1	1	—	—	—
273	—	—	—	1	—	1	—
278	—	—	—	—	—	—	—
283	—	—	—	—	—	—	1
N	55	23	15	6	1	1	1
Mean (cm.)	1·93	2·11	2·33	2·53	2·54	2·75	2·84

TABLE 10. *VENUS STRIATULA*. HEIGHT/LENGTH AND THICKNESS/LENGTH RELATIONSHIP, KAMES BAY

Mid point length	No. of specimens	Mean length (cm)	Mean height (cm)	Mean thickness (cm)	Height/length	Thickness/length
0·75	4	0·779	0·668	0·362	0·858	0·465
0·85	10	0·886	0·744	0·417	0·840	0·471
0·95	10	0·967	0·810	0·469	0·838	0·485
1·05	10	1·053	0·881	0·502	0·837	0·477
1·15	10	1·131	0·922	0·548	0·815	0·485
1·25	10	1·253	1·045	0·639	0·834	0·510
1·35	10	1·369	1·158	0·685	0·846	0·500
1·45	10	1·465	1·249	0·749	0·853	0·511
1·55	10	1·548	1·313	0·810	0·848	0·523
1·65	10	1·650	1·403	0·846	0·850	0·513
1·75	10	1·761	1·490	0·930	0·846	0·528
1·85	6	1·875	1·588	0·982	0·847	0·524
1·95	10	1·940	1·647	1·040	0·849	0·536
2·05	9	2·041	1·695	1·044	0·830	0·512
2·15	5	2·158	1·804	1·120	0·836	0·519
2·25	1	2·240	1·930	1·130	0·862	0·504

TABLE 11. *VENUS STRIATULA*. GROWTH AND MORTALITY AT DIFFERENT ARTIFICIAL POPULATION DENSITIES

Population density per $\frac{1}{10}$ m^2	Mean length (cm)	Mean length R1 (cm)	% growth	% mortality
10	1·22	0·99	23·2	0
30	1·29	1·05	22·9	6·67
30	1·19	1·01	17·8	6·67
100	1·23	1·04	18·3	9·0
100	1·24	1·02	21·6	8·0
200	1·26	1·05	20·0	6·67
500	1·26	1·08	16·7	—
1000	1·25	1·06	17·9	7·0
Kames Bay	1·21	0·98	23·5	—

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

ATKINS, D., 1960. The development of the pleopods in the young crab stages of *Pinnotheres* (Crustacea). *Proc. zool. Soc. Lond.*, Vol. 133, pp. 435–51.

In female *Pinnotheres pinnotheres* (L.) and *P. pisum* (Pennant) the four pairs of pleopods present in the megalopa persist, undergoing modification to form those of the adult.

In male *P. pinnotheres* in addition to pleopods 1 and 2 (the copulatory appendages), pleopods 3–5 of the megalopa persist in degenerate form in crabs of 4 mm carapace width, and pleopods 3 and 4 in crabs 6 mm wide. The exopodite of pleopod 2 is recognizable in large males of carapace width 7 mm.

In male *P. pisum* pleopods 3–5 disappear early, pleopod 3 only being present in a male of 1·40 mm carapace width, but absent in one of 1·44 mm carapace width. The exopodite of pleopod 2 is absent in males from a carapace width of 3·5 mm. D.A.

BONE, Q., 1960. The origin of the chordates. *J. Linn. Soc.* (*Zool.*), Vol. 44, pp. 252–69.

This consideration of chordate origins re-states the case for the neotenic origin of the chordate line from the larvae of primitive hemichordates. It is assumed that the ancestral semi-sessile hemichordate group possessed a tadpole-like larva as the distributory phase in their life-cycle, and that the gradual neoteny of this larva led to the chordates and the acraniates. The urochordates are derived from the ancestral group by the modification of the adult and the retention of the larval stage, the larval stage becoming modified for short range site-selection upon the adoption of an absolutely sessile habit. The larval stages of the acraniates and of modern hemichordates are regarded as of secondary origin, consequent upon the adoption of a more completely sessile habit than at the origin of each group, and upon the paedomorphic changes which have been assumed to have taken place in the history of the modern hemichordates. These conclusions are reached from a consideration of the types of larvae which are found in the modern representatives of the groups discussed; from a consideration of their adult organization; and from a consideration of the functional problems involved in the neotenic transformation which is supposed to have resulted in the origin of the chordate line. The paper is followed by a discussion, in which the views put forward are criticized by protagonists of other theories of chordate origins. Q.B.

BONEY, A. D., 1960. Observations on the spore output of some common red algae. *Brit. phycol. Bull.*, Vol. 2, pp. 36–7.

The spore output of six common red algae was measured. Estimates of the spore output per 1 g fresh weight per hour indicated that plants making a shorter annual appearance on the shore tended to have a greater spore output than those with more protracted fruiting periods; the output of plants producing larger spores also tended to be low. A count made of all the tetrasporangia on a plant of *Plumaria elegans* (preserved weight 6 g) gave a total of 47,387, of which 62·3 % were ready to release their spores, 16·4 % were developing, 20·3 % had liberated their spores, and 0·8 % were abortive. A.D.B.

BONEY, A. D., 1960. The spore output of *Antithamnion plumula* over a number of days. *Brit. phycol. Bull.*, Vol. 2, pp. 37–8.

A fruiting tuft of *Antithamnion plumula* (dry weight 0·0082 g), produced 10,071 tetraspores over a 6-day period, 51·7% of the spores being liberated in the first 24 h. A plant of dry weight 1 g might be expected to produce 1·25 million spores over the same time interval.

A.D.B.

BONEY, A. D., 1960. Nurture of a fruiting *Antithamnion* tuft and the physiological condition of the liberated spores. *Brit. phycol. Bull.*, Vol. 2, pp. 38–9.

Sporelings grown from spores which had developed in sporangia borne by branches cultured in Erd–Schreiber medium showed a growth rate which was twice that of sporelings grown from the spores produced by sporangia of branches cultured in filtered sea water.

A.D.B.

CALDWELL, P. C., 1960. The phosphorus metabolism of squid axons and its relationship to the active transport of sodium. *J. Physiol.*, Vol. 152, pp. 545–60.

The effects of cyanide, 2-4-dinitrophenol, azide and ouabain on the phosphate compounds in giant axons from the squid, *Loligo forbesi*, have been studied. 2 mM cyanide, 0·2 mM dinitrophenol and 3 mM azide each bring about a decline in the amounts of the 'high energy' phosphate compounds (arginine phosphate and ATP) and there is some recovery when the inhibitor is removed. Since these inhibitors are known to block the sodium efflux of cephalopod giant axons, it is suggested that the decline in the 'high energy' phosphate compounds may be connected with the blocking of sodium transport. 10^{-5} M ouabain, which is also known to block the sodium efflux, does not, however, appear to have an effect on the 'high energy' phosphate compounds.

P.C.C.

CALDWELL, P. C., HODGKIN, A. L., KEYNES, R. D. & SHAW, T. I., 1960. The effects of injecting 'energy-rich' phosphate compounds on the active transport of ions in the giant axons of *Loligo*. *J. Physiol.*, Vol. 152, pp. 561–90.

Injections of ATP, ADP, GTP, arginine phosphate and phosphoenol-pyruvate (PEP) caused a transient increase in the efflux of labelled sodium from squid giant axons which had been poisoned with cyanide. Injections of creatine phosphate, arginine, AMP, inorganic phosphate and other compounds were ineffective as were ATP and arginine phosphate when applied externally.

The number of Na ions extruded was roughly proportional to the number of energy-rich phosphate groups injected, the Na/~ P ratio for arginine phosphate, ATP and PEP being about 0·7.

High concentrations of arginine phosphate and PEP restored the normal K-sensitivity of the Na efflux but ATP, ADP and GTP did not.

Injections of arginine phosphate but not ATP caused a substantial increase in the uptake of potassium in fibres poisoned with cyanide.

It is concluded that the extrusion of sodium depends on a supply of energy-rich phosphate groups and that the presence of arginine phosphate as well as ATP is important.

P.C.C.

CALDWELL, P. C., HODGKIN, A. L., KEYNES, R. D. & SHAW, T. I., 1960. Partial inhibition of the active transport of cations in the giant axons of *Loligo*. *J. Physiol.*, Vol. 152, pp. 591–600.

Under conditions when a squid giant axon should contain ATP but not arginine phosphate the sodium efflux is not reduced by removing external potassium and is higher than the efflux from an unpoisoned fibre into a K-free solution. A transient rise in the Na efflux into a K-free solution is seen in the early stages of cyanide poisoning and a sustained rise occurs if the axon is treated with 0·2 mM-DNP at pH 8. Since K-sensitivity can be partly restored by injecting arginine phosphate but not ATP it is concluded that the normal coupling between K and Na movements may depend on the presence of arginine phosphate.

P.C.C.

CARLISLE, D. B., 1960. Softening chitin for histology. *Nature, Lond.*, Vol. 187, pp. 1132–33.

Chitin may be softened for sectioning, without damage to other tissues, by the enzyme chitinase, extracted from mushrooms or puffballs.

D.B.C.

ENDEAN, R., 1960. The blood-cells of the ascidian, *Phallusia mammillata*. *Quart. J. micr. Sci.*, Vol. 101, pp. 177–97.

An investigation has been made of the structure, histochemistry and histogenesis of the blood-cells of *Phallusia mammillata* (Cuvier).

Vanadocytes, pigment cells, phagocytes and cells with acid vacuoles arise from primitive lymphocytes. A series of transition stages can be recognized in each case. During the development of each cell type, there is a progressive reduction in nuclear volume and RNA appears to be released from the nucleus.

About 98 % of the total number of blood cells are vanadocytes or their precursors. A number of globules is possessed by each vanadocyte. Each globule possesses a refractive surface membrane containing compound lipid and a core containing a vanadium compound, protein, H_2SO_4, and carbohydrate material. Part of the carbohydrate material is in a dispersed form and can be hydrolysed with 2 N-H_2SO_4. The remainder is in the form of birefringent grains and appears to be a very insoluble polysaccharide. The carbohydrate material may be the precursor of the tunicin of the test.

The pigment cells contain melanin and oriented submicroscopic crystallites.

R.E.

LEON, Y. A., BULBROOK, R. D. & CORNER, E. D. S., 1960. Steroid sulphatase, arylsulphatase and β-glucuronidase in the Mollusca. *Biochem. J.*, Vol. 75, pp. 612–17.

Acetone-dried powders prepared from thirteen species of molluscs have been tested for steroid-sulphatase activity with the sulphates of dehydro*epi*androsterone, androsterone and aetiocholanolone as substrates.

Three species (*Helix pomatia*, *Buccinum undatum* and *Nassarius reticulatus*) were found to possess activity towards both dehydro*epi*androsterone sulphate and aetiocholanolone sulphate, whereas in four species (*Patella vulgata*, *Littorina littorea*, *Patina pellucida* and *Nucella lapillus*) activity towards only dehydro*epi*androsterone sulphate was found. Steroid sulphatase activity was not detected in six species.

No enzyme capable of hydrolysing androsterone sulphate was found in any of the

animals tested; therefore complete enzymic hydrolysis of urinary steroid conjugates cannot be achieved with enzyme preparations from these molluscs.

β-Glucuronidase and arylsulphatase activity are widely distributed in the Mollusca, but the activities of these enzymes are not related to the activity of steroid sulphatase.

MURRAY, R. W., 1960. The response of the ampullae of Lorenzini of elasmobranchs to mechanical stimulation. *J. exp. Biol.*, Vol. 37, pp. 417–24.

Electrical recording shows that the ampullae are sensitive to weak tactile stimuli ($<$ 1 mg) applied to the ends of their tubes. The response may be either increase or decrease in the resting frequency, with an opposite after-effect. Adaptation is rapid, but this may be due to accommodative changes of the tissues. Possible functions are discussed. R.W.M.

MURRAY, R. W., 1960. Electrical sensitivity of the ampullae of Lorenzini. *Nature, Lond.*, Vol. 187, p. 957.

The nerve impulse discharge from the ampullae of Lorenzini of rays is sensitive to changes in the voltage gradient in the surrounding water of 1–2 μV/cm. The impulse frequency increases when the gradient lies along the tubes, openings negative. Adaptation occurs to maintained stimuli. Possible functions are suggested. R.W.M.

MURRAY, R. W., 1960. Initiation of cutaneous sensory nerve impulses. *Proc. physiol. Soc.*, 22–23 April 1960. *J. Physiol.*, Vol. 152, 53–54*P*.

The impulse discharge from single cutaneous fibres in rays has been recorded without dissection by a wire electrode on the skin surface. Mechanical and electrical stimuli are effective. The details of the recorded potentials suggest that propagated impulses are initiated subterminally in the nerves. R.W.M.

NICHOLS, D., 1960. The histology and activities of the tube-feet of *Antedon bifida*. *Quart. J. micr. Sci.*, Vol. 101, pp. 105–17.

The histological structure of the tube-feet of this crinoid is interpreted functionally. The epithelium of each tube-foot is raised in places into papillae, each of which contains mucous glands, a single muscle fibre and terminal nerve processes. In a feeding animal the tube-feet are exceedingly active, each one making periodic rapid bending movements towards the food-groove of the arm or pinnule; it is suggested that the tube-feet act to produce a mucous food-trap by the throwing action of the tube-feet combined with the contraction of the papillal muscles serving to squeeze out the mucus. In the absence of ampullae, protraction of the tube-feet is likely to be brought about by contraction of muscle fibres stretched across the water-vascular canal, the volume of which will be decreased at the expense of the underlying perihaemal canal. D.N.

RALPH, P. M., 1960. *Tetraplatia*, a coronate scyphomedusan. *Proc. roy. Soc.*, Ser. B, Vol. 152, pp. 263–81.

The true relationships of the pelagic coelenterate *Tetraplatia* have long been a matter of controversy. The most recent and widely held view, that it is a highly modified trachyline medusa, is here demonstrated to be untenable because it has no velum, it

has gastric filaments, a sense organ that is a rhopalium with an endodermal statolith, and gonads that originate in the endoderm. A reinvestigation based on adequate samples shows that it is a scyphomedusan.

It is suggested first that *Tetraplatia* has no ephyral stage and the planula develops directly into a juvenile, and secondly that *Tetraplatia* evolved from a coronate scyphomedusan in which the normal radial expansion of the ephyra larva was arrested at an early stage and replaced by accelerated growth in the oral-aboral axis. Later, or accompanying the increased growth rate on the oral-aboral axis, four pairs of pouches were formed, causing displacement and crowding of the eight ephyran lappets into four pairs and finally their fusion into a single locomotory organ. Thus, there were four locomotory lappets separated by four pairs of pouches. *T. volitans* remains essentially unaltered from this basic pattern, but in *T. chuni* the pouches fuse to form the well known flying buttresses.

Tetraplatia should now be placed in the coronate Scyphomedusae, near the Ephyropsidae and Periphyllidae, in a family of its own, the Tetraplatidae. For this study, material of both *T. volitans* and *T. chuni* has been available. P.M.R.

Ross, D. M., 1960. The association between the hermit crab *Eupagurus bernhardus* (L.) and the sea anemone *Calliactis parasitica* (Couch). *Proc. zool. Soc. Lond.*, Vol. 134, pp. 43–57.

Preliminary observations on *Calliactis parasitica* and *Eupagurus bernhardus* in experimental containers suggested that, at Plymouth, the crab plays no part in attaching the anemone to the shell. This contrasts sharply with the descriptions of the association between *Calliactis* and hermit crabs in the Mediterranean. In Plymouth, the anemone transfers itself to a shell by a complicated manoeuvre, in which the adhesion of the tentacles, the detachment of the base, the swinging of the base to the shell, and the subsequent settling on the shell and resumption of an upright posture, follow each other in a co-ordinated sequence of orderly movements, taking 15–30 min to complete.

Experiments with empty shells and shells occupied by crabs showed that *Calliactis* transfer with equal frequency in both cases, although individual anemones show a wide variation in the tendency to transfer to shells of both kinds. The anemone settles on shells of living *Buccinum*, although not found thereon in nature, and does not desert these for empty shells or for shells occupied by crabs. These activities of *Calliactis* in response to shells were not observed when the shells offered had been cleaned of organic matter by boiling in alkali. Comparing the accounts of this association in the Mediterranean and Atlantic habitats points to a number of interesting problems of comparative ecology and behaviour. D.M.R.

BOOK REVIEW

The Biology of Marine Animals

By J. A. Colin Nicol

Sir Isaac Pitman and Sons Ltd., London, 1960. Pp. xi + 707, with 294 text-figures. Price 95 *s*.

There is nothing else quite like this in biological literature. Dr Colin Nicol has produced a book for which all students of marine biology and all workers in that wide field must be very grateful. It is so compacted of carefully selected and compiled information, so well documented with invaluable reference lists, so well provided with figures, graphs and tables that there is hardly a marine biologist who can afford to be without it. The publishers therefore may well contend that it is worth its very high price; the buyer may feel that he has been held up to ransom.

The writing of this book has taken several years; an immense devotion has gone into its making. Those of us who have observed Dr Nicol at work in the library inevitably became aware of the meticulous care with which he was extracting the essential parts of a vast array of original papers. We can only thank him for a book which will be a constant companion for years to come and for a textual clarity rare in pages so closely packed with factual information as are these.

Dr Nicol's theme is the whole of life in the sea. Beginning with the watery environment he passes naturally to a consideration of body fluids and circulation systems, thence to respiration and nutrition. Feeding mechanisms, digestion and excretion follow. Sense organs, nervous systems, their mechanisms and the behaviour which they occasion continue the story. In these fields the author has himself made noteworthy contributions to science. But the longest list of references to his own papers occurs after the chapter on luminescence, a subject in which of recent years he has almost more than anyone else become a pioneer. Pigments, colours and colour changes are discussed immediately before luminescence; these are matters which inevitably attract attention when the phenomenon of luminescence escapes unobserved and are even less understood. The chapter on animal associations (commensalism, parasitism and symbiosis) gives a first class cross-section of modern knowledge and thinking on that subject. The last chapter deals with skeletons, shelters and special defences. There is an appendix on saline media and a comprehensive index.

D. P. W.

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THE OCEANOGRAPHY OF THE CELTIC SEA

I. WIND DRIFT

By L. H. N. Cooper, D.Sc.
The Plymouth Laboratory

(With Text-fig 1)

In the Celtic Sea, an extensive shallow area bordering the eastern North North Atlantic in 50° N. lat., we need to know the pattern of the currents, what drives them and what they carry. Our climate is evidence that much warm water is brought to our shores, so that we are concerned with an embayment of what is loosely called the Gulf Stream.

Dr J. N. Carruthers has for long stressed the importance of wind in all studies of marine productivity and has initiated and maintained, with the help of many colleagues, current measurements at light vessels, to be correlated with wind. In the southern North Sea these light vessels are spread over the whole area, but in the Celtic Sea there are relatively few and these are placed where they cannot reflect the current system even a few miles away. We have seen that when the measurements at the Seven Stones Light Vessel were generalized for the whole of Land's End passage they were misleading, although hidden within them was the basis of understanding to be extracted (Cooper, Lawford & Veley 1960). Again, currents at the Coningbeg Light Vessel will in large measure reflect the shape of the Saltees rather than events in the Celtic Sea. Consequently the circulation of the central Celtic Sea cannot be studied by current measurements at light-vessels as in the central North Sea.

Though the circulation of the whole of the North Atlantic is wind driven there is very strong intensification of currents on the western side where they persist without regard to local winds. The part played by the earth's rotation in bringing about this western intensification is becoming better understood, largely due to work by Stommel and by Munk (Stommel, 1958). On theoretical grounds Munk has been able to devise a system of gyrals which closely represent events. There is a boundary between two gyrals in about 50° N. lat., so that if we had no local winds hereabouts and our local circulation was mainly due to ocean-wide causes we should have only trifling currents.

There is, however, a North Atlantic drift in a generally north-easterly or easterly direction towards the British Isles and Faroe–Shetland Channel. Within the Celtic Sea most current charts show arrows pointing east towards the English Channel, or with a clockwise curl parallel with the Biscayan coasts of France and then of Spain. Though this is the predominating current system

resulting from the prevailing winds, at any moment currents created within the Celtic Sea by variable winds may be found flowing in any direction (*Irish Pilot*, Hydrographic Department, Admiralty, 1954). Over the year as a whole, and in most months, winds from south-west and west are more frequent than from any other direction, the most frequent directions after south-west and west being south and northwest. From March to May, by contrast, north-easterly winds become frequent while northerly winds are common in April. However, preceding our cruise in April 1950 (Cooper, 1961), northerly or north-easterly winds did not occur until we were actually running along the south coast of Ireland. Informative wind roses for the Western English Channel and the Irish Sea have been published by Dietrich *et al.* (1952).

Prevailing winds must create prevailing drift currents. In winter our prevailing south-westerly and westerly winds should produce a surface drift of about 45° to the right (Ekman, 1905). Our prevailing surface currents should therefore set towards east or south-east in winter and will be reflected by the drift of ships and surface-dwelling animals.

The empirical direction given in the *Irish Pilot* is 30° to the right of the wind. This is probably a mean statement derived from the drift of ships in all 12 months and from observations from survey ships, mostly made in summer.

The transfer of energy from the surface downwards takes time, so that transient changes in direction of surface drift should be smoothed out. The movement of deeper water, though much less in velocity, should be more constant in direction. It is this deeper drift that we have some hope of recognizing when we attempt to derive residual water movements from the distribution of properties such as temperature and salinity.

Mass transport in winter will tend to be 90° to the right of the prevailing winds in the Western Celtic Sea, i.e. towards south-east and south. As the Celtic Sea shoals towards the east, mass transport in winter will come more nearly in line with the surface drift, i.e. towards Ushant and the south-western English Channel.

In summer, when a strong thermocline builds up, wind drift will become confined to the warm surface layer and will approach more nearly to the direction of the wind.

These three paragraphs, written at the very end of the investigation, epitomize the empirical findings and agree with the requirements of the Ekman spiral.

They mean that wind records from the well-exposed Meteorological Station at Scilly, in conjunction with the Ekman spiral, may be used to give a rough and ready assessment of wind drift in the Celtic Sea well away from land at the time of a specific biological investigation. A further advantage of concentration of effort at Scilly is that there have already been a number of meteorological and current studies there (*inter alia*, Sheppard, Charnock & Francis, 1952; Carruthers, Lawford & Veley, 1951*b*).

They also mean that, if there is no change in the direction of the prevailing wind, the North Atlantic wind drift both over the deep ocean and within the Celtic Sea should take a more northerly direction in summer than in winter. Again the warmer the surface water, the more northerly the summer deviation will be.

The strandings of exotic animals on our coasts, such as Kemp's loggerhead turtles (Deraniyagala, 1939), *Physalia* (Wilson, 1947) and *Ianthina* (Wilson & Wilson, 1956) provide evidence about the north-easterly drift in the North Atlantic, but do not clarify our understanding of circulation within the Celtic Sea.

This account does not apply near coasts so that we have to search for restricted regions of the Celtic Sea where a more persistent and predictable system of currents may flow (Cooper, 1960*a*, *b c*). Since the word 'current' has come to acquire a wide and diffuse range of meanings, we will first define what it is to mean here. Physicists commonly have precise meanings in mind, made clearer by suitable adjectives and use of the notion of streamlines (cf. Lamb 1924, p. 18, and Milne-Thompson, 1938, p. 273). The streamlines set out in an earlier paper on water flow west of Ushant in summer (Cooper, 1960*b*) conformed with these limitations. The currents associated with such streamlines involve little turbulent mixing with enveloping waters and the chemical properties of water along the length of such currents will change little due to physical causes. The chemical properties may change due to photosynthesis and metabolic processes of organisms living in the water.

When measurements are made at a fixed position, such as a light vessel, the word 'current' has a different meaning. Each measurement singly is a measurement of current, but over a period of time very different water masses from very different sources may pass by. The sum of all the measurements does not make up a current in the sense first considered. This argument applies whether or not we first 'extract the tide'. With Carruthers *et al.* (1951*b*) it is better here to speak of 'residual water movement'.

The eddy 'current' of the north-eastern Celtic Sea, to be discussed, is something different again. Though such a system of water movements does not conform with a physical picture based on streamlines, nevertheless, it must involve considerable conservation of energy and momentum. It is subject to exchange of heat with the atmosphere and of turbulent mixing with neighbouring waters. There is great variation in chemical and biological properties around the eddy so that it may be termed a current only in a diffuse sense. Separate sectors of the eddy will be described as the Land's End corner current, the South Wales coastal current, the Carnsore corner current and the Nymphe Bank current.

Movement of surface water by variable winds must necessarily lead to much exchange of water between water masses and to much turbulent mixing. Though in a somewhat statistical sense a residual current results, it is utterly

different in character from a streamlined affair such as has been described for the intermediate and bottom water west of Ushant in summer (Cooper, 1960*b*). That had nothing to do with the local winds.

I prefer therefore not to use the precise word 'current' for the phenomenon observed in the western Celtic Sea in early spring but to describe it by the vaguer word 'drift'.

Evidence is slowly accumulating (cf. p. 263) that the movement of water in such a drift may be intensified over the break of slope between the shelf and the continental slope of the Celtic Sea. Application of the term 'current' to this feature, if indeed it persists over a distance, may be misleading since it might suggest the power of long-distance transport of animals. It seems more likely that such water, with the animals it carries, would frequently be slung to one side and replaced by new water with another population of animals. Unless and until streamline flow is established in currents above the continental edge it would be wise to assume its absence. There may be continuity of movement but not of matter.

In quantum theory it is impossible to regulate or measure the co-ordinates and momenta of a system more accurately than to require that they lie somewhere within a given cell. Although for different reasons, there is a similar limitation upon biological applications of non-streamlined water movements. One may say that, when studying transport of chemical constituents or biological organisms by a non-streamlined system of water movement, it is not practical to measure the co-ordinates and momenta of the system more accurately than to require that those lie somewhat within a given parcel of water of fairly large size.

ON COMPUTING WIND DRIFT FROM WIND STRESS

The wind stress at the surface τ is usually related to the wind velocity W by an equation due to Ekman (1905) and much studied by Thorade (1914)

$$\tau = \gamma^2 \rho' W^2, \tag{1}$$

where ρ' is the density of the air; γ^2 is a dimensionless constant but dependent upon the height at which the wind is measured, upon the nature of the stability of the moving air and upon the surface of the sea being hydrodynamically rough with a roughness length of 0·6 cm (Rossby, 1935; Rossby & Montgomery, 1935)

This means that equation (1) may hold for winds exceeding 5 m/sec (12 knots, or force 4 Beaufort and over). Winds of Beaufort force 3 and less would seem to have little power to produce wind stress sufficient to initiate much water movement. A number of investigations (Sverdrup, Johnson & Fleming, 1942, pp. 489–91) have agreed that γ^2 has a value close to 0·0026 and today there is no more assurance about this value (Fofonoff, 1960).

Associated with equation (1) and subject to the same limitations is a simple equation for calculating wind drift v from wind velocity W.

$$v = \frac{kW}{\sqrt{\sin \phi}}, \tag{2}$$

where ϕ is the latitude.

In view of the doubt that still remains today as to the value of k it is remarkable that the presently accepted value 0·0126 differs so little from that first proposed by Ekman (1905), 0·0127. In our waters it has been found to fit observations at a number of light vessels (Carruthers, Lawford & Veley, 1950*a*, *b*,; 1951*a*, *b*; Carruthers & Lawford, 1950).

Nevertheless, in a careful study, spanning 7 days in January, 1951 at Scilly (Sheppard *et al.* 1952), the conditions of air flow required for equations (1) and (2) to hold were not found.

Unfortunately, though Munk (1947) has agreed with Ekman (1905) and Thorade (1914) as to the validity of the two equations, others have proposed that the wind stress may be proportional to $W^{\frac{3}{2}}$ (Neumann, 1948) or W^3 (Francis, 1951). Evidently the conditions of stability or turbulence of air flow has a large effect on the relations between wind drift, wind stress and wind velocity.

For practical application, it would seem that we must accept equation (2) as a semi-empirical means of assessing wind drift from meteorological records.

MUGGIAEA AS A BIOLOGICAL INDICATOR OF WIND DRIFT IN THE CELTIC SEA

The siphonophore *Muggiaea atlantica* was first identified in Plymouth waters, and used as a drift indicator by Gough (1905). Russell (1934; 1935*a*, *b*) considers it to be an indicator of 'south-western' water. It may rapidly reproduce asexually, and, since it seems to favour the upper layers, it is an indicator of wind drift.

Extensive records of its distribution in the Celtic Sea were made during the mackerel investigations in 1937–9. In 1938 between 7 and 18 April and 31 May and 5 June (Corbin, 1947, p. 67, fig. 1; p. 107, fig. 13D and E), from the neighbourhood of Land's End it appeared to spread to the south-west. This direction of movement was not what would have been anticipated from our picture of the 'prevailing' drift current in the mouth of the English Channel (Matthews, 1914).

Consequently in 1947 I estimated the probable wind-drift current at the time of Corbin's investigations from the recorded winds at Scilly. Though recorded continuously with an anemometer at 10 m, these results were reported in the Daily Weather Report as Beaufort numbers.

The records have been reconverted by the standard formula (Meteorological Office, 1952)

$$W \text{ (in cm/sec)} = 83{\cdot}6\sqrt{B^3}. \tag{3}$$

In 49° N. lat, combining equation (2) and (3)

$$v = 1{\cdot}32\sqrt{B^3}. \tag{4}$$

Assuming that the wind blows steadily with force B Beaufort for 3 h before and 3 h after the time of the reading, the distance travelled by the surface drift in 49° N. would be $0{\cdot}285\ B^{\frac{3}{2}}$ km.

The wind does not blow for long with unchanged strength, neither does it continue precisely in one direction; none the less there are periods when it blows from one general direction for some time so that the time spent in initiating the drift is small compared with the time spent in maintaining it. When this happens, as in April and May 1938, it is possible to break down the wind records into consecutive groups. Within each group the computed wind drifts have been added vectorially (Table 1), but equating winds of force 0–3 to zero drift.

TABLE 1. WIND DRIFT AND DISTRIBUTION OF *MUGGIAEA*

Wind drift computed between two cruises on which the distribution of *Muggiaea atlantica* was charted, 1938.

Period	11 April (21.00 h) to 8 May (03.00 h)	8 May (03.00 h) to 15 May (15.00 h)	15 May (15.00 h) to 1 June (21.00 h)
Total number of records	105	30	69
Records of light breezes, force 0–3	34	14	25
Records of force 4–8	71	16	44
Days elapsing	26½	7½	17½
Vector mean direction of winds force 4–8, from	52°	187°	278°
Assumed deviation of wind drift from direction of wind	+40°	+35°	+30°
Direction of wind drift, towards	272°	52°	128°
Vector wind drift (km)	166	44	104
Scalar wind drift (km)	189	47	139
Efficiency factor	0·88	0·93	0·75
Depth of frictional influence (m)	65	74	72

If the wind blows steadily in a given direction the maximum proportion of the wind energy will be employed in moving the water in a straight line. If the direction and strength vary somewhat, the drift will change in direction so that the distance made good in a straight line will be less than the actual zig-zag course followed, i.e. the vector distance will be less than the scalar distance. Moreover, some of the available energy of the wind will be spent in overcoming inertia when changing the direction of the drift and not for urging this forward; about 24 h will be required to develop a new steady drift after a change of wind (Ekman 1905, p. 16). The ratio of the vector to the scalar distance therefore provides a measure of the efficiency of the winds in

setting up a drift. A prolonged steady wind would have an efficiency factor of unity, whilst winds completely random in direction and strength would have an efficiency factor approaching zero. In Table 1 assessments of this factor are included and it is suggested that greater trust can be put in the final drift calculation if the factor exceeds 0·9 than if it had been only, say, 0·5.

An exact assessment of the angle of deviation of the mean vector drift from the mean vector wind is not possible. About 11 April the water had just ceased to be vertically homogeneous so that the surface skin of water would

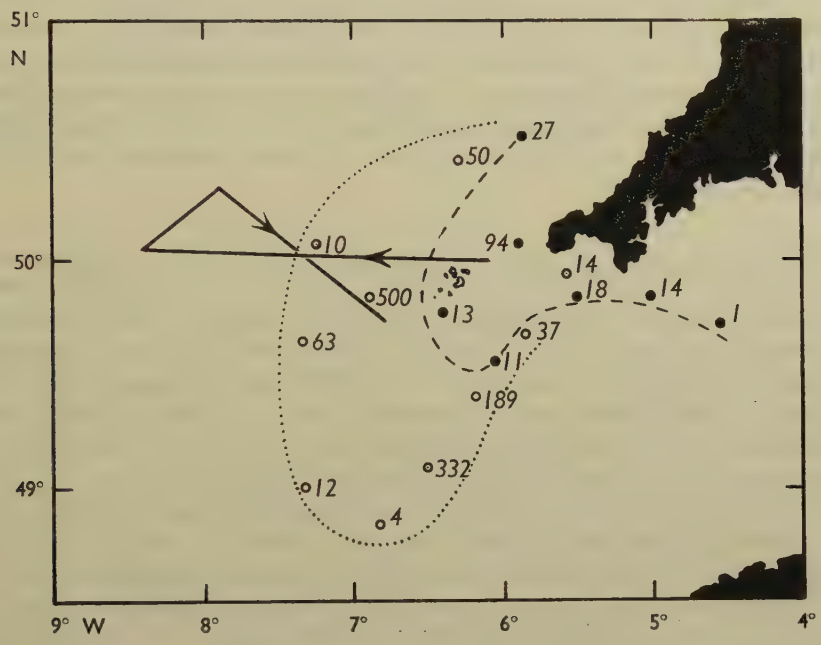

Fig. 1. Extension of the distribution of *Muggiaea atlantica* by wind drift in April–May 1938. Solid circles show positions and numbers of the animal observed in a standard 2 m stramin ring net (young fish trawl) between 7 and 16 April 1938, open circles between 31 May and 6 June. On both occasions the blank area of the chart was covered with a sufficiency of zero records. The course of the computed surface drift between the two cruises is shown.

have deviated, perhaps, 40° to the right of the wind and the mass transport of the water containing *M. atlantica*, somewhat more. By 1 June a thermocline should have developed and the empirical direction, 30° to the right given in the *Irish Pilot*, more likely applies.

In evaluating the wind drift of the water carrying *M. atlantica*, the deviations given in Table 1 have been assessed for the three periods. It will be seen that the dispersion of the population of the siphonophore south-west from Land's End agrees with this calculated wind drift (Fig. 1). The two methods check each other.

Thus (*a*) the semi-empirical equation (2) provides a means of computing the magnitude of the wind drift from wind records; (*b*) the assessed deviations of the wind drift cannot be very far wrong; (*c*) within their own catches marine ecologists may find the means of assessing wind drift.

Working in 1948 it had been deduced somewhat intuitively, that the prevailing south-westerly winds over the Celtic Sea were likely to initiate a current setting south or south-west from the neighbourhood of Carnsore Point and Waterford Harbour. Also it was thought that the bottom topography in the south-western and central parts of the Celtic Sea was such as to encourage currents to flow towards north-east or south-west (Cooper, 1960*a*; and Cooper & Vaux, 1949, fig. 3). Papers have already been presented on the flow west of Ushant (Cooper, 1960*b*) and between Land's End and the Scilly Isles (Cooper, 1960*c*; and Cooper, Lawford & Veley, 1960). Cruise results in the northern Celtic Sea in 1950 will be discussed in another article (this *Journal*, pp. 235–270).

ON THE DRIFT OF PLANKTON IN THE CELTIC SEA

A population of animals having the same compressibility as sea water and without power to swim would be carried, together with all other properties of the water, along a streamline such as may exist below the thermocline in the western English Channel at high summer. Such a population would have little tendency to disperse due to turbulent movement of the water so that its movement should be susceptible to a strictly quantitative study.

Many plankton animals are vertical migrants and may spend the hours of darkness in the upper waters which need to be considered separately. Here, also, no doubt, a redistribution of mass is occurring for the same reasons as in the deep water but it cannot be studied by the same techniques. Density, however, is continuously but irregularly suffering change due to irradiation and exchange of sensible heat with the atmosphere. The sea surface drift also is being induced and modified by the wind. All of the wind-drift layer is above the thermocline across which, when well developed, little wind stress seems to be transmitted. It is possible to calculate from known winds what such a wind drift is likely to amount to; indeed such calculations are likely to be nearer the truth than deductions from the distribution of temperature, salinity and mass.

Even so, providing that the biologist will provide the physical oceanographer with a detailed life history, particularly as to probable vertical distribution, of each animal of interest, it should be possible to make a reasonably probable estimate of its movements. In strong contrast in a drift, such as that of the north-western Celtic Sea in early spring, driven by variable winds, there is a large degree of randomness in all that happens. The forces which tend towards dispersion are large compared with those that tend to-

wards streamline motion. Studies of the distribution of properties or, for that matter, information from current meters, can only measure a statistical trend.

The value of statistical assessment of drift may be improved if it can be combined with a very full knowledge of the habits and life history of an organism. Thus, if we know that an animal spends its whole life in the uppermost 10 m of water, then its future travel may be best assessed by computing vectorially the wind drift from the observed winds. Deductions from temperature and salinity are likely to be misleading. If, however, we know that the animal spends its whole life over the shelf in water deeper than, say, 50 m, the best the physical oceanographer can do is to assess the residual wind-induced deep drift on the basis of temperature, salinity or any more direct means available. Direct measurements of current are likely to be of value only if there are many of them from an appropriate depth.

SUMMARY

The dependence of prevailing wind drift in the Celtic sea upon prevailing winds is discussed and a proposal made to use wind records for the Scilly Meteorological Observatory for assessing drift currents for use in biological investigations.

If there is no seasonal change in the direction of the prevailing winds, nevertheless, the wind drift in summer will be more northerly than in winter.

The terms 'current', 'residual water movement' and 'drift', as used in this series of papers, are defined. The Ekman–Thorade equation for computing wind drift from wind is discussed and applied to the drift of the biological indicator *Muggiaea atlantica* in 1938. The two approaches agree to suggest that either method may be applied in biological distribution studies.

Some effects of drift, due to variable winds, on plankton distribution are outlined.

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THE OCEANOGRAPHY OF THE CELTIC SEA

II. CONDITIONS IN THE SPRING OF 1950

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(With Text-figures 1–18)

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In April 1950 a cruise was undertaken on R.V. 'Sabella', commanded by Lt.-Cdr. C. A. Hoodless, D.S.C., R.N.R., to learn more of the currents around Land's End, St David's Head and Carnsore Point and to test some of the hypotheses presented earlier (Cooper, 1960*a*).

Much evidence in that month (Cooper, 1960*c*) has shown that in the southern Celtic Sea south of the 50th parallel, the water was moving towards east or somewhat south of east. This is in exact agreement with the generalized surface current circulation illustrated in the regional Admiralty Pilots (Cooper, 1961). In spite of this (Cooper, Lawford & Veley, 1960) it was clear that earlier, probably during preceding periods of neap tides, there had been considerable transport from south to north of Cornwall by way of a narrow, strong, but intermittent current setting snugly around Land's End. In April and in June this movement of water was established by quite different

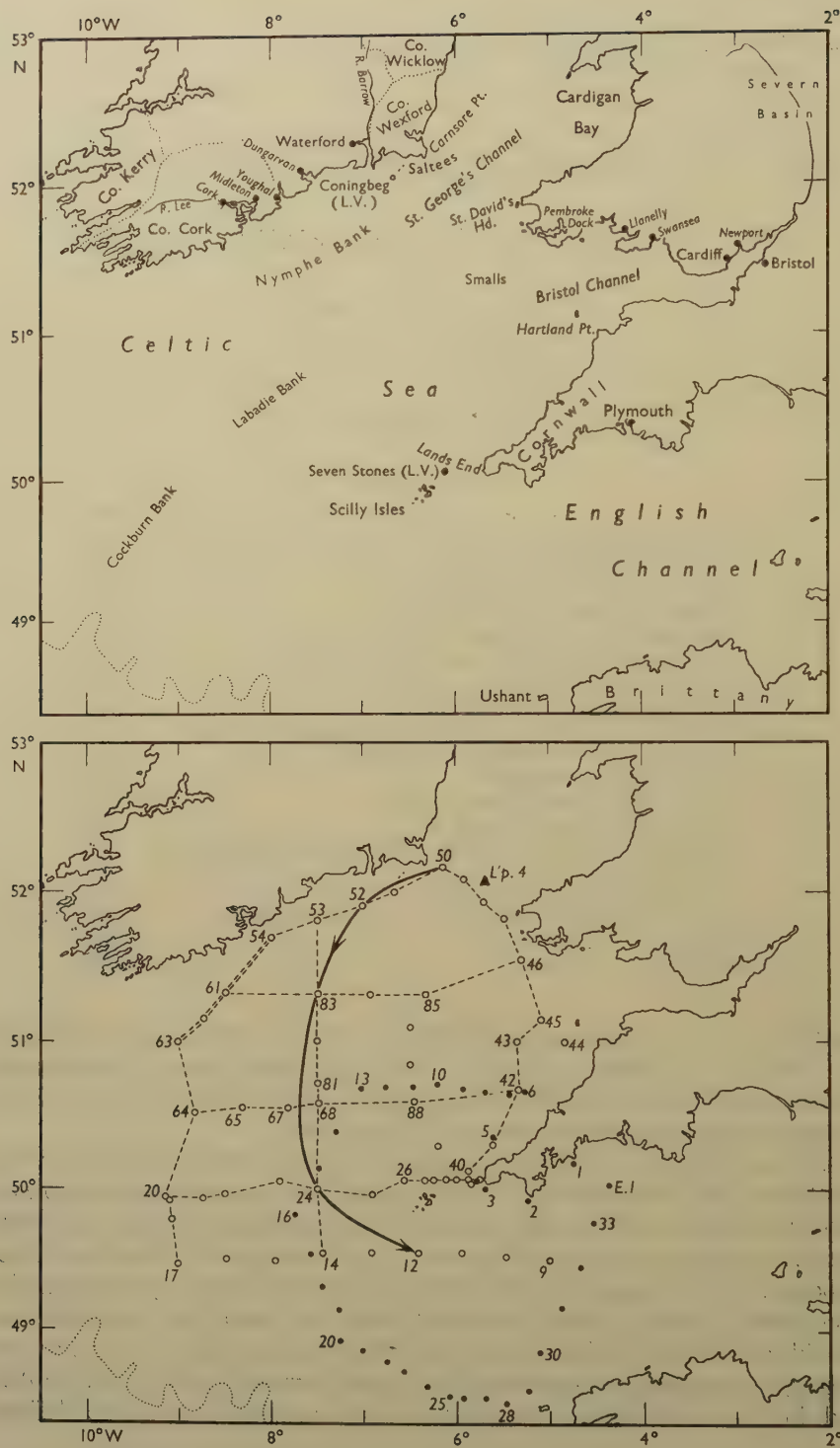

Fig. 1. Top: the Celtic Sea with place names used here. Bottom: open circles, stations 18–30 April 1950; solid circles, stations 12–16 June 1950; pecked lines, sections shown in Figs. 3, 5, 6, 8–12 (and also Cooper, 1960*c*, figs. 3 and 4); solid line, approximate course of the Nymphe Bank current in April. Sections for the June cruise have already been published (Cooper, 1960*b*, fig. 15; and 1960*c*, figs. 8, 9, and 10).

arguments (Cooper, 1960*a*, fig. 4; 1960*c*). This water north of Cornwall will here be called Land's End water.

The station positions are set out in Fig. 1. All fixes on R.V. 'Sabella' were astronomical, by land-marks or by dead reckoning. The observations have been published (Conseil International, 1954).

The pattern of winds over the Celtic Sea in the month preceding the cruise of April 1950 has already been published (Cooper, 1960*a*, table 1), together with the surface drift, likely to result from these winds (fig. 1, panel S.W). Again the further effect of the structure of the land mass of southern Ireland was there presented as fig. 3. The channeling effect on wind of the incised river valleys running west–east is likely there to initiate an offshore set towards south-east or south-south-east.

Nowhere in the observations presented was there evidence that in April 1950 there was any water heavy enough to have contributed to a cascading system (Cooper & Vaux, 1949).

GEOPOTENTIAL TOPOGRAPHIES

The geopotential topographies of Fig. 2 have to be interpreted with great care. There can nowhere be a surface of no motion and since the currents are wind-induced, a change of wind will at once start to change the current. Even when, as in April, 1950, the pattern of winds was predominantly westerly and south-westerly there were considerable variations in strength and direction. Consequently the wind drift can never become streamlined and fully equilibrated with the distribution of mass. None the less, when such topographies suggest a current required by a study of continuity of properties, the confirmation is valuable. When they suggest the opposite, they *may* indicate that transport was mostly in a subsurface current, or a change in the current system due to a change in wind.

Only around St David's Head do the topographies depict an equilibrium state, the South Wales coastal current in full flow. There is an indication of the Carnsore Point corner current. South of Ireland the topographies suggest the impossible, water running into the land mass of Ireland. Consequently we have to seek for compensation currents to get the water away again. Indeed, we shall produce evidence for strong up-welling off Waterford, but this had been created by northerly winds which only started to blow while the cruise was being run. The up-welled waters had not approached dynamic equilibrium and, since the cruise took 10 days, the chart is not strictly synoptic and does not show the effect. The chart gives a reasonably clear picture of the current from the Nymphe Bank as far as the parallel 50° 30′ N.

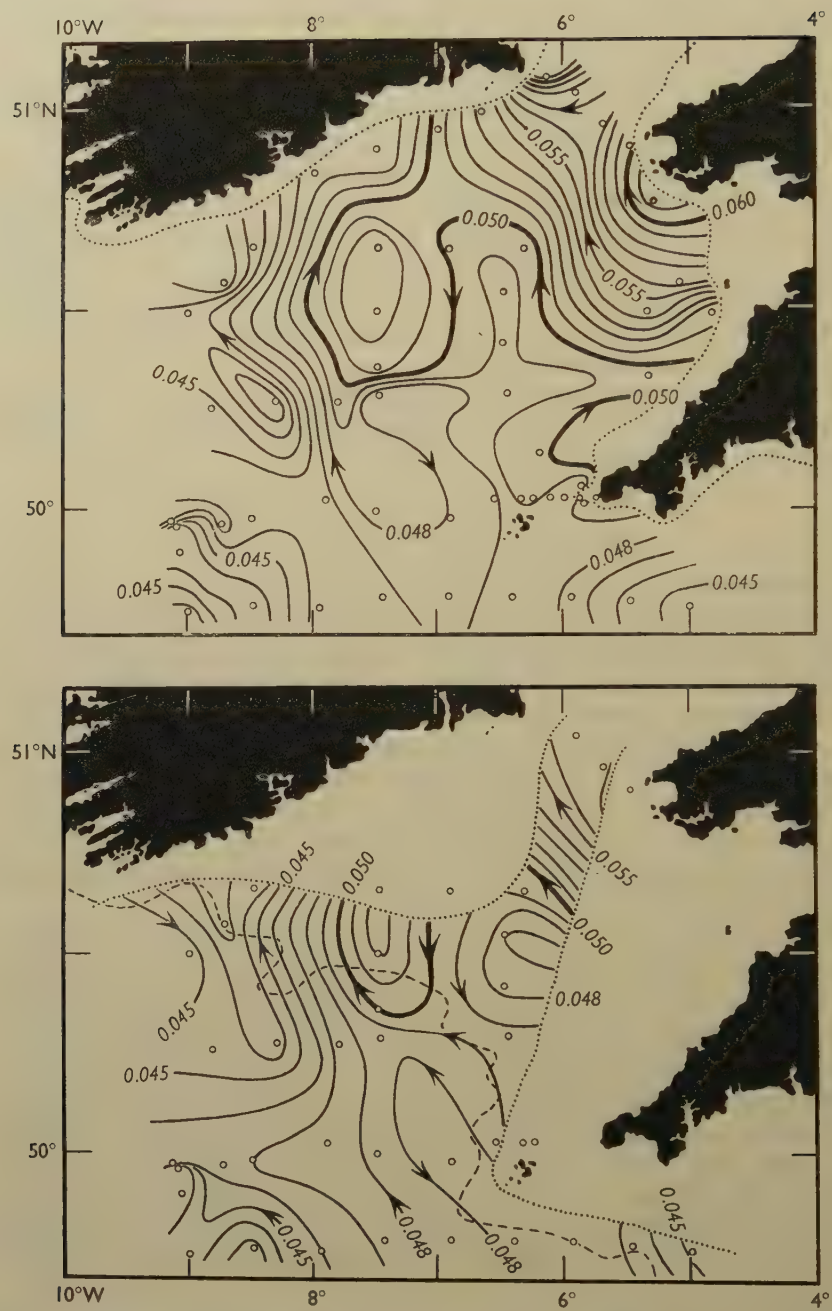

Fig. 2. Geopotential topographies April 1950, top 0–50 m, bottom 50–100 m. The arrows indicate direction of surface motion relative to the reference level. In some cases, as south of Cork, they should be read reversed to indicate motion of the reference level compared with the surface.

EVIDENCE FOR WARMING-UP IN THE CELTIC SEA

When comparing other years with 1950 one must consider the effect of short- and long-term climatic change. The Irish Fisheries vessel, S.S. 'Muirchu', under the direction of the late Mr G. P. Farran, made quarterly cruises to standard positions south of Ireland. Many of these cruises were made between 1920 and 1933 and they stopped in 1936. Bowden (1950, Appendix) has prepared tables of mean temperatures and salinities for nine of these stations. Three of these were incorporated in our cruises on 26–29 April and may be compared with Bowden's May averages for depths below the thermocline (Table 1).

TABLE 1. WARMING-UP SOUTH OF IRELAND

The year 1950 compared with a quarter of a century earlier.

Latitude	Longitude	Irish station	'Sabella' station	Rise in temperature (°C)
51° 40′ N.	8° 00′ W.	A	54	+0·50°
50° 40′ N.	8° 00′ W.	D	65/67	+0·20°
51° 00′ N.	9° 00′ W.	9B	63	+0·61°

Differences in salinity, being within ±0·02‰, call for no comment. The temperatures show that the deeper water was 0·2–0·6° warmer than 20 years earlier, in spite of our cruise being ahead of the month in which the Irish standard observations were made. This is consistent with the findings at the Seven Stones Light Vessel.

THE MOUTH OF THE BRISTOL CHANNEL

In the mouth of the Bristol Channel several types of water have been recognized (Fig. 3), which will be considered in turn.

Land's End water

The properties of the water north of Cornwall carried in early in April by the Land's End corner current have already been described (Cooper, 1960*c*, table 4, figs. 4 and 8, tables 6 and 7: positions U and V). Its properties in June (1960*c*, fig. 10) and the boundary with northern Celtic Sea Water will not be again discussed.

South Wales coastal water

This water, lighter than σ_t 26·9 less saline than 34·8‰, cooler than 9·5° C and turbid, was found off the coast of Pembrokeshire at stations 47 and 46 and covered much of the Smalls fishing ground. It was relatively rich in phosphate, total phosphorus and silicate (Table 2). Calculation suggested that the diluting fresh water brought in about 10 μg-atom phosphorus per litre, most of this being inorganic. Domestic and industrial sewage from

South Wales and leaching from the rich farmlands of the Severn Basin seem to be the source of this enrichment. The large catchment area of the Severn Basin draining to the Bristol Channel is depicted by Bowden (1950, fig. 1) and its run off is described by Lee (1960).

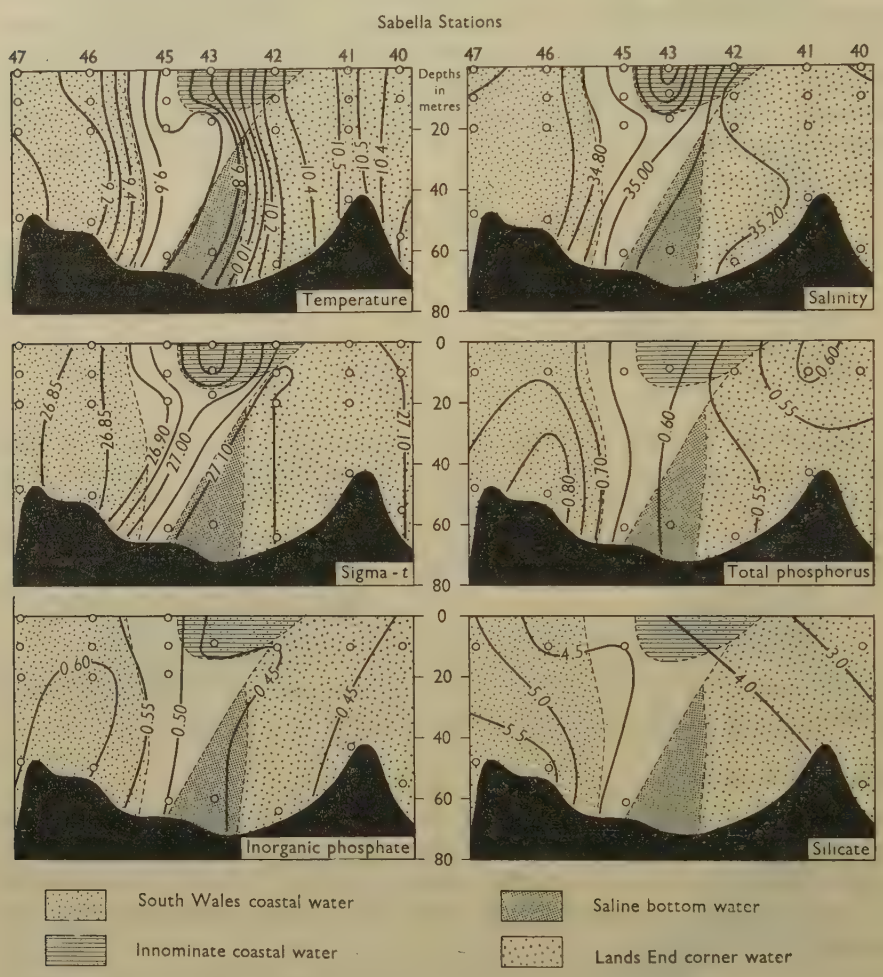

Fig. 3. A section from off St David's Head (station 47) to Land's End (station 40), 23–25 April 1950.

I had always assumed that nutrients discharged into the sea as sewage would be immediately available to plants and that a phytoplankton bloom would be followed in a few weeks by large populations of zooplankton which would then attract pelagic fish. The findings in the South Wales coastal water have seemed at variance with this postulate.

Only station 46 was sampled for plant pigments which, compared with the resources and the date, proved to be poor, 110 Harvey units/m³. The 2 m young fish trawls made with post-war stramin do not effectively sample the copepod herbivores. By station 48, a considerable population of carnivores was building up, but, since station 46 was 40 miles from Llanelly, the nearest considerable town and more than 80 miles from Bristol, Cardiff and Newport whence came much of the phosphate, there would seem to have been plenty of time for a large micro-plankton population to have built up. During the

TABLE 2. DISTRIBUTION OF NUTRIENTS IN SOUTH WALES COASTAL WATERS, 25 APRIL 1950

Station and sounding	Sample depth (m)	Phosphorus (μg-atom/l.) Total	Inorganic: In unfiltered water	Inorganic: In filtered water	Particulate	Organic	Silicon $-SiO_3$	Plant pigments Harvey units/m³
46	0·5	—	0·56	0·54	0·02	—	—	110
(58 m)	10	0·74	0·57	0·56	0·01	0·17	4·4	—
	20	—	0·62	0·56	0·06	—	—	—
	50	0·85	0·60	0·54	0·06	0·25	5·6	—
47	0·5	—	0·58	0·56	0·02	—	—	—
(90 m)	10	0·72	0·58	0·54	0·04	0·14	5·2	—
	20	—	0·59	0·54	0·05	—	—	—
	48	0·77	0·60	0·54	0·06	0·17	5·7	—
48	10	0·74	0·60	0·54	0·06	0·14	5·2	—
(110 m)	20	0·64	0·60	0·56	0·04	0·04	5·2	—
	48	—	0·57	—	—	—	—	—
	81	0·75	0·59	—	—	0·10	5·4	—

last 10 years I have repeatedly sought an understanding of these anomalous results and failed to find one. A remarkable parallel has now been published for the Hyperion sewage scheme of the City of Los Angeles by Stevenson & Grady (1956), whose results have been quoted by Emery (1960, p. 138). The content of phosphate in the effluent there is 1000 times its concentration in average surface sea water (p. 301). However, collections of plankton showed only a slight average increase of dinoflagellate populations, uncertain increases of diatoms, copepods, tunicates, tintinnids, and annelid larvae, and no increase of other forms. No blooms have there been related to sewage discharge. Abundant heavy metals discharged in sewage (as is likely from the Swansea area) had no identifiable effect on the sea water or its life. Since it is of much economic importance that the expensive nutrients discharged in sewage shall be recovered by the fisheries, this confirmation of failure quickly to do so by an investigation far fuller than ours is relevant.

The Los Angeles workers comment on the turbidity of sea water which has received sewage discharge. The turbidity of the samples drawn from the three stations in South Wales coastal water was notably great, so much so that for these stations and only for these, duplicate phosphate samples were

filtered before analysis as shown in Table 2. Possibly it is this turbidity which so far reduces available light as greatly to slow down the rate of photosynthesis. If turbidity is not the cause, it may be necessary to search for an antibiotic in the effluent.

We have seen that along the coast of South Wales the drainage, largely urban, had introduced about 10 μg-atom inorganic phosphate per litre of fresh water and that we know nothing of the growth-affecting substances which may accompany this added phosphate. The littoral animals and plants of Pembrokeshire would then be bathed by water markedly different from that of North Cornwall. It would be of value to the hydrographer to know whether the flora and fauna of the coasts of Pembrokeshire differs in species and in total numbers from the coasts of North Cornwall under similar conditions of exposure and substratum.

The boundary between the warm saline water on the Cornish side of the Bristol Channel and the cold brackish water on the Welsh side sloped downward from south to north with a gradient of 0·2 % (cf. Uda, 1959) (Fig. 3). The gradient of the bevel dividing the two currents is likely to change with the state of the tide and the phase of the moon. For future development of this problem it will be necessary to know that on 23 April 1950, station 42 was worked 1 h before and station 43 2¼ h after high water at Pembroke Dock.

Innominate coastal water

There was a detached surface bubble of brackish water in mid-Channel at station 43 (T, 9·85°; sal., 34·66–34·74‰). Its relatively low content of phosphorus compounds suggests either that it had been the site of a plant outburst or, more probably, that it had come from the English side of the Bristol Channel somewhere east of Hartland Point.

Saline bottom water

At station 43 (below 20 m), at the bottom at station 45 (Fig. 3), and at the extra station 44 off Hartland Point there was a wedge of heavy saline water. It was much too cold (mostly 9·65–9·75° C) to have come from south of Land's End in the immediately preceding weeks. Alternative possible origins are presented so that future work may be designed to choose between them. (*a*) The water had entered from the west along the 51st parallel of latitude from the seventh meridian to coalesce with the east-flowing Land's End water; this seems more probable (Table 3), but (*b*) it is not impossible that it had been formed during the coldest months of the winter from Land's End water which had invaded the shelf (mostly < 16 fm or 29 m) on the English side between Hartland Point and Watchet. On this shallow shelf it had been cooled to cascade into the deep channel where it became the southernmost and deepest part of the west-flowing South Wales current.

The nutrient contents are not truly diagnostic since there are bottom muds available to enrich waters from either source. In this position the distribution of mass based on too few observations and almost certainly subject to strong tidal displacement is considered a treacherous guide.

A definitive answer is much needed since if the bottom current is moving west, it is likely to be the carrier of the muds which build up the deposits around the 51st parallel still further west.

TABLE 3. PROPERTIES OF BRISTOL CHANNEL BOTTOM WATER

Station no.	86	87	43	Mean
Latitude (° N.)	51° 07′	50° 53′	51° 00′	—
Longitude (° W.)	6° 30′	6° 30′	5° 20′	—
Sounding, (m)	99	95	73	—
Depth sampled (m)	85	80	60	—
Temperature (°C)	9·57	9·77	9·75	9·7
Salinity (‰)	35·16	35·17	35·14	35·16
Density (σ_t)	27·16	27·14	27·13	27·14
Total phosphorus (μg-atom/l.)	0·63	0·60	0·59	0·61
Inorganic phosphate (μg-atom/l.)	0·44	0·44	0·46	0·45
Organic phosphorus (μg-atom/l.)	0·19	0·16	0·13	0·16

Other water

When these four water types have been recognized, there remained a body of water not stippled in Fig. 3. This was akin to the South Wales coastal water but was more saline, warmer and poorer in nutrients. However, it had little in common with the Land's End water. It evidently had spent some time within the Bristol Channel and was unquestionably moving west.

ST GEORGE'S CHANNEL

On the centre line is 'station 4', maintained by the Department of Oceanography in the University of Liverpool (Figs. 1, 4). Its most striking feature is the much greater variability of temperature and salinity than at the Seven Stones Light Vessel. The salinity was much lower in the summer than in the winter months. How the distribution of nutrients and plankton correlate with these variations is not known.

The water on 25–26 April in the St George's Channel, compared with the Celtic Sea, was cool and of low salinity (Fig. 5, stations 50–47). In the middle of the channel lay water with a salinity of about 34·66‰. This low salinity presents a problem. It is well established (Bowden, 1955) that the general movement of water in the Irish Sea is from south to north but if this water is recruited directly from the Celtic Sea its salinity would be expected to be much higher than it commonly is in summer. On a single occasion such a low salinity could be ascribed to a short-lived eddy, but it is difficult to explain the prolonged low-salinity water at 'station 4' between April and August 1950 (Fig. 4) in this way. It is easier to believe that the whole width of St George's Channel, except that occupied by the narrow Carnsore corner current, was

filled during these months with water from the northern Bristol Channel and coast of South Wales, no water coming directly from the Celtic Sea.

In the first week of September conditions changed at 'station 4' where salinity increased rapidly and remained relatively high during the autumn. This change is in line with accepted principles. During the summer months the coastal waters of South Wales had become lighter than those of the Bristol Channel further from shore, due to the effects of dilution by run off and warming up in shallow waters, particularly in sandy bays. Dilution makes for

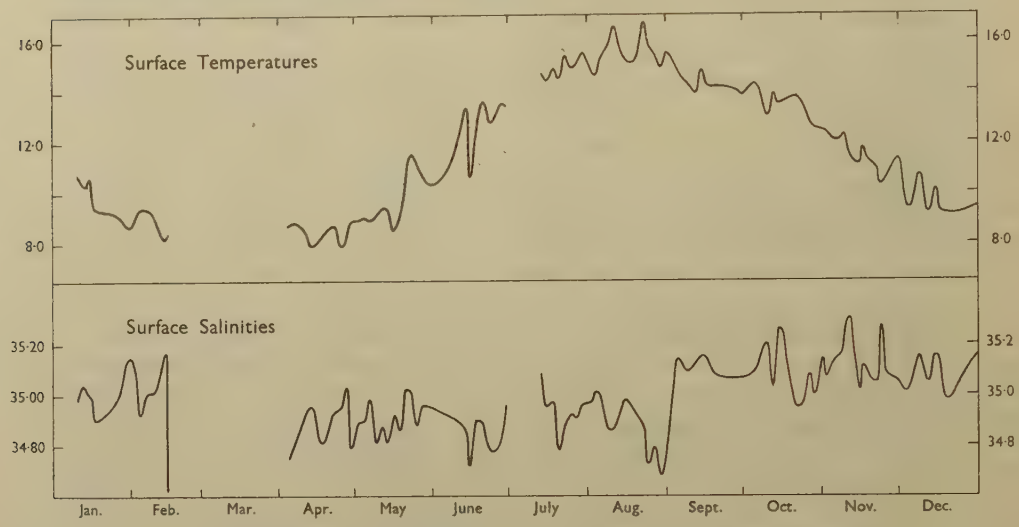

Fig. 4. Temperatures and salinities in 1950 at Liverpool University Station 4, 52° 04′ N, 5° 40′ W.

lighter water and a current running the whole year round with the coast on the right, but, as soon as autumn sets in, shallow coastal waters cool faster than deeper waters where the same heat loss is distributed through a greater quantity of water. When the increase in density due to cooling exactly offsets the decrease in density due to dilution no coastal density current should flow. Something of this kind seems to happen to the South Wales coastal current in autumn: as a result the position of 'station 4' may then become filled with more saline water more directly recruited from the Celtic Sea. Also during early September 'thermocline recruitment' into the Bristol Channel of the kind already described for the English Channel (Cooper, 1960*b*) may perhaps occur. If this account is correct, the consequences for the local climate and for the biological populations of West Wales may be notable (cf. Cooper, 1960*c* for the case of north Cornwall in summer).

The silicate content of the water on the South Wales side (station 47) was twice that on the Irish side (station 50). Though the two coastal streams had

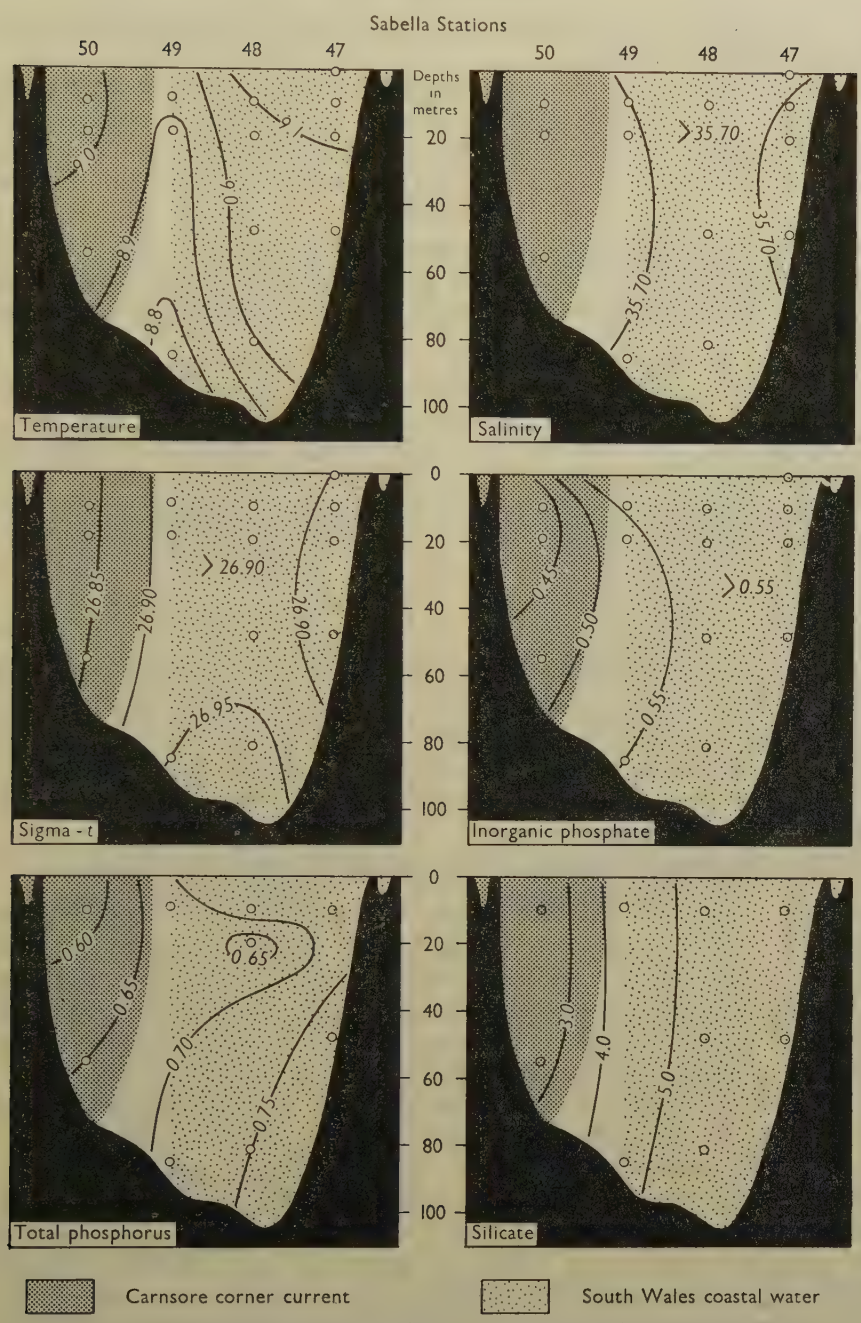

Fig. 5. Section from Carnsore Point to St David's Head, 25–26 April 1950. In the salinity diagram *for* '35.70' *read* '34.70'.

similar temperatures and salinities, they did not have a common origin. There is no possibility of an eddy.

The Carnsore corner current, southbound and probably quite narrow, carried one component of the Nymphe Bank current whose course is to be deduced (Table 4). The drainage from the hard Palaeozoic rocks of County Wicklow and County Wexford evidently contributed little but distilled water to the Carnsore corner current. The South Wales coastal water received water not only from a similar mountain drainage area but from the industrial and domestic drainage from South Wales and Monmouthshire plus the drainage from the lush, heavily fertilized agricultural lands of the Severn Basin.

TABLE 4. PROPERTIES OF THE COMPONENT WATERS OF THE NYMPHE BANK CURRENT, APRIL 1950

	Carnsore corner current	County Cork coastal water	Waterford mud-enriched water
Temperature (°C)	9·0°	9·4°	9·1°
Salinity (‰)	34·61	35·1	34·95
Density (σ_t)	26·83	27·15	27·08
Total phosphorus (μg-atom/l.)	0·61	0·5	0·71
Inorganic phosphate (μg-atom/l.)	0·45	0·35	0·61
Organic phosphorus (μg-atom/l.)	0·16	0·2	0·10
Silicate (μg-atom/l.)	2·4	—	—

If the contrast in nutrient content on the two sides of St George's Channel, revealed in April 1950, is always true, then it would have much importance for any study of productivity. It is significant that none of the 1180 drift bottles put out by Daniel (Daniel & Lewis, 1929) left the Irish Sea by the Carnsore corner current. The section across St George's Channel needs frequent repetition.

THE COASTAL WATERS OF SOUTHERN IRELAND

As far as station 51, two miles south of Coningbeg Light Vessel, the ship remained in the very poor water which had come around Carnsore Point. At the next station, 52, lying off Waterford Harbour and in line with the straight incised valley of the Barrow, the salinity and temperature showed that we were still in this Carnsore corner current water but total phosphorus and inorganic phosphate had become very high (Fig. 6).

For the 72 h preceding this station the wind at Midleton, situated east of Cork in the wind-gap extending the valley of the river Lee, attained a vector mean speed of 15 knots from a direction 337°. Since the geomorphological limitations of Midleton as a wind-observing station are very similar to those of Waterford Harbour, it is a fair deduction that there also the wind was 'such as to initiate an offsetting surface current' (Cooper, 1960*a*, fig. 3) compensated by local up-welling of water much enriched by breakdown of the bottom muds. The inference is that during northerly and north-westerly winds, a rich and productive water mass may be created from

a poor one, leading ultimately to a temporary abundance of pelagic fish. This seems to be a key area for the nutrient oceanography of the Celtic Sea so that the process needs further study.

The more westerly stations 53 and 54 were occupied by County Cork coastal water, established on the basis of the geopotential topographies (Fig. 2,

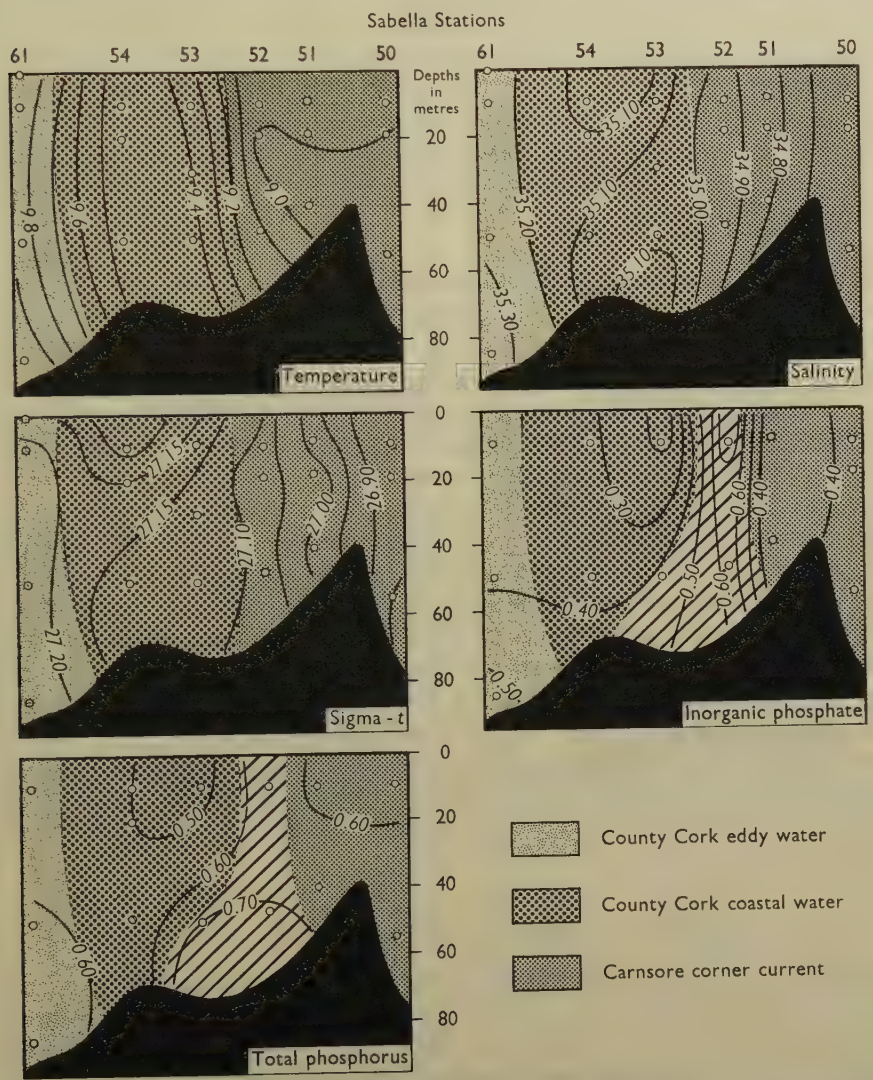

Fig. 6. Section along the south coast of Ireland, 26 and 28 April 1950. Stippled as shown. Cross-hatching in the panels for inorganic phosphate and total phosphorus indicates the area where northerly winds channelled by the valley of the Barrow caused up-welling of bottom water enriched with compounds of phosphorus from the bottom muds thereabouts.

p. 238) and the area distribution of properties. It was not established that this somewhat high salinity water bathed the cliffs. It was probably, as a wind drift, flowing east towards Youghal and Waterford, where it was forced to turn offshore by the pressure of the north eastern Celtic Sea cyclonic circulation (Cooper, 1960*a*, pp. 160–2).

The total phosphorus contents of the bottom water at stations 52 and 53 off Waterford and Dungarvan provided evidence for the thesis that the muddy bottom hereabouts was the site of liberation of about 0·15 μg-atom/l. phosphorus (cf. Cooper, 1951). This then up-welled off Waterford. This conclusion rests on too few analyses to be accepted without reserve but is in accord with the earlier hypothesis and is supported by a small temperature inversion. The bottom water was 0·1° C warmer than the top 20 m, suggesting an indraught from the south.

Residual water movement at Coningbeg Light Vessel

On admiralty Chart 2049 the rate of the tidal stream is given at a point 3 miles S.S.W. from Carnsore Point and at the Coningbeg Light Vessel. Evidently the residual current remains to be extracted. At the Carnsore Point position it is 3·1 miles per tidal cycle at springs towards 259°. Averaged over a lunation this gives an average current of 4·5 miles per sidereal day towards 259°. This suggests that the Carnsore corner current is a continuing affair.

At the Coningbeg Light Vessel the residual water movement extracted from the tidal stream observations is 1·43 miles towards 210° per tidal cycle at springs, and 0·75 miles towards 207° at neaps. Averaged over a lunation this represents a residual water movement of 2 miles a sidereal day towards 209°. This lines up with the direction of the Nymphe Bank drift which has been deduced from our own observations. However, the residual water movement at Coningbeg need be no more than a local physiographic consequence of the shape of the Saltees (Fig. 1), which could induce an off-shore set towards south-south-west on both flood and ebb tides. Whatever the explanation may truly be, these tidal stream data reinforce the view that the region around the Saltees and Carnsore Point is a critical one for the oceanography of the Celtic Sea.

THE CURRENT SETTING SOUTH FROM THE NYMPHE BANK

We have seen that in April 1950 a very mixed water seems to have been created south of Waterford by fusion (Table 4) of cold, low-salinity, nutrient-poor water, which had travelled the south-east coast of Ireland to enter the area as a corner current around Carnsore Point, with warm high-salinity water from the offing of County Cork. A core of water within this water mass, but not all of it, had been enriched by up-welling of water containing breakdown products from a veneer of bottom mud.

The chart of geopotential topography of the zero against 50 decibar surface has been presented in Fig. 2 (p. 238). A current around Carnsore Point sets on to the Nymphe Bank and then to the south for 100 miles. It had had two branches, one being controlled in some measure by the Labadie Bank.

Naturally the median course of the current does not set precisely through the positions of stations worked, but a section constructed on the basis of

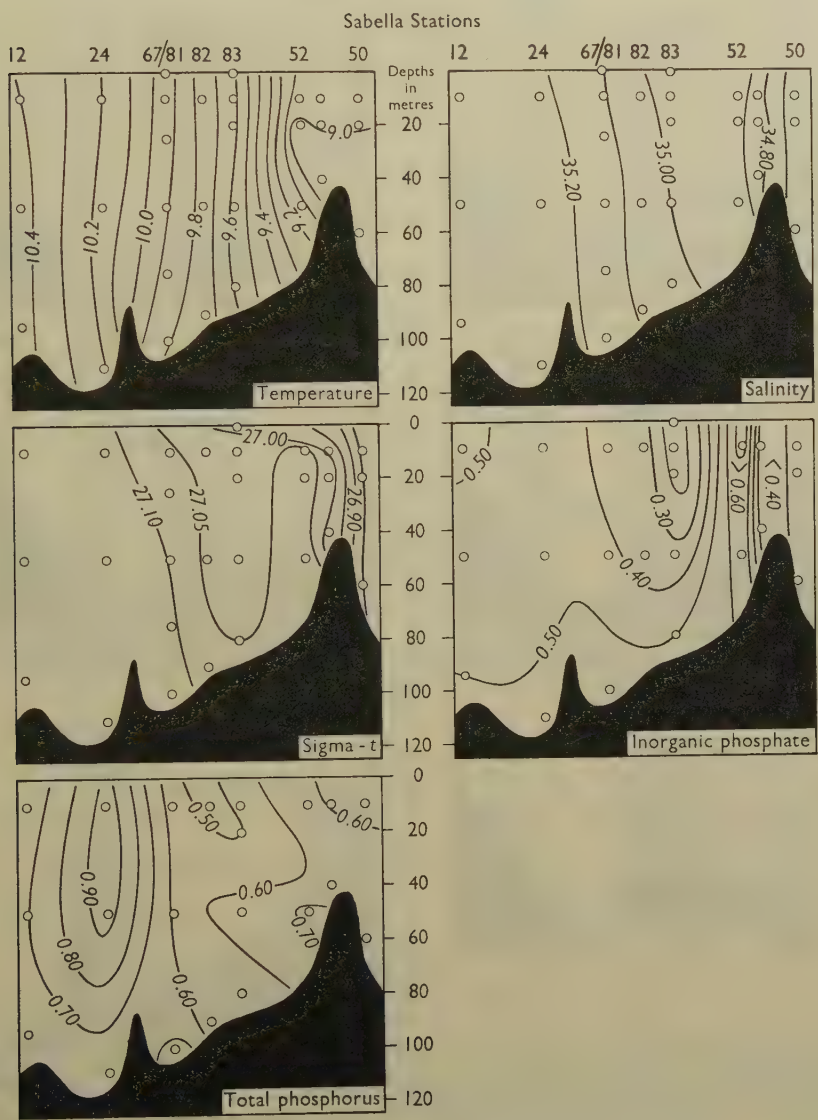

Fig. 7. Properties along the axis of the Nymphe Bank current.

minimum temperature and minimum salinity from the nearest stations to this course is presented in Fig. 7. Starting from a region with a depth of about 60 m, by the time it reached station 12 the water had warmed up by 1·4° C, due to irradiation and mixing. Salinity increased by 0·5‰. The current

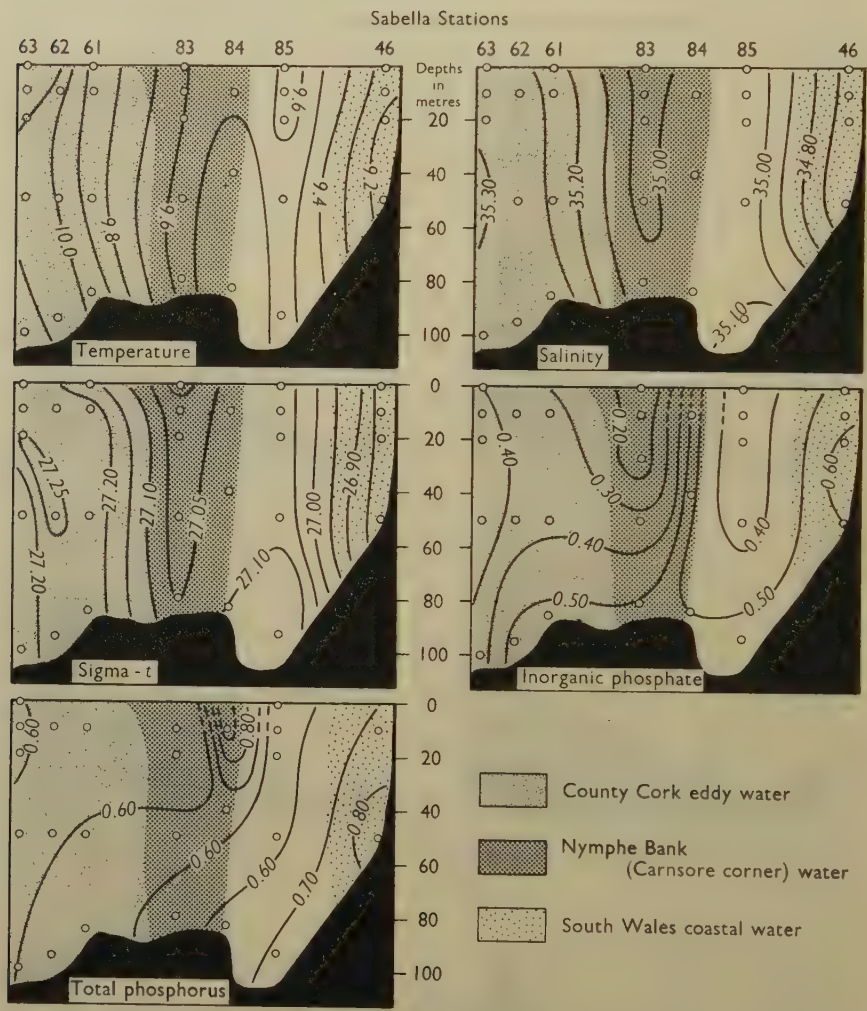

Fig. 8. Section along the parallel 51° 20′ N. lat.

included streamlines or 'schlieren' of the several waters built into it, some phosphate-poor, others enriched by up-welling off Waterford. This rather than plant production was probably the reason why compounds of phosphorus were non-conservative.

To deduce the course of the Nymphe Bank current in April 1950 sections along three parallels of latitude and two meridians have been selected (Figs. 8–12).

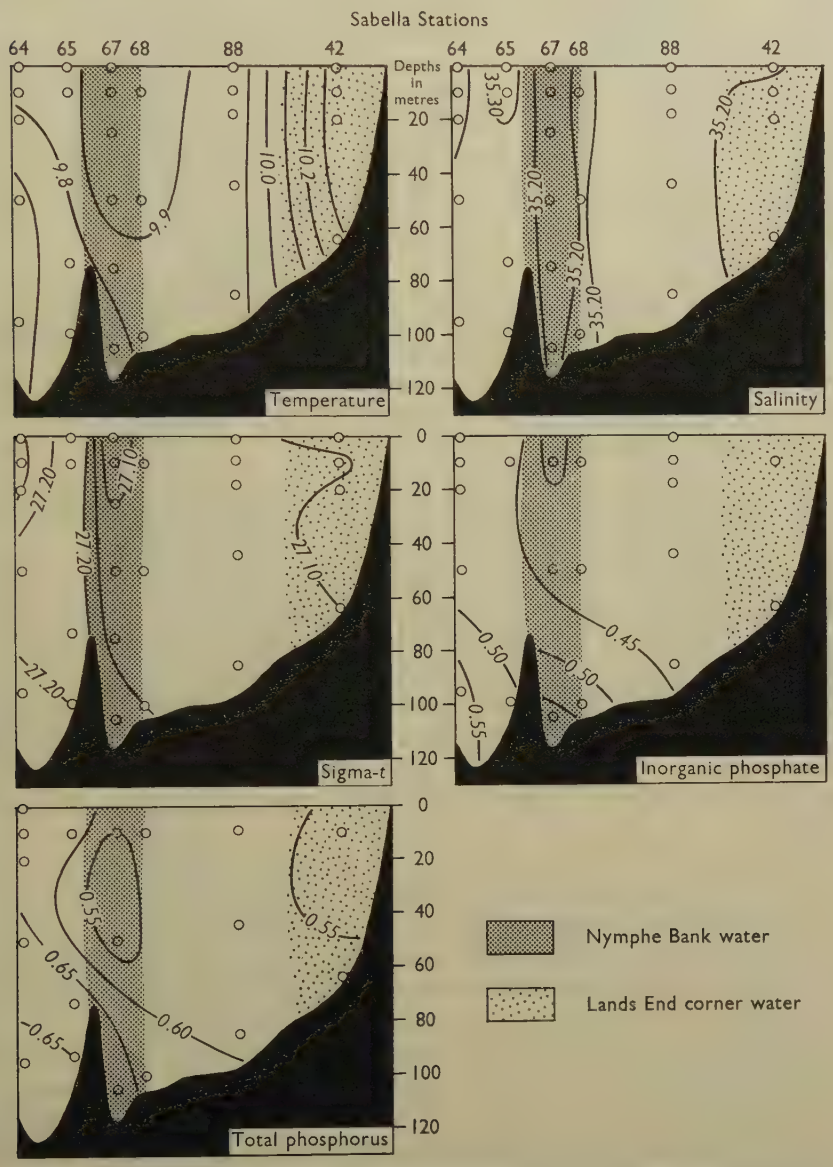

Fig. 9. Section along the parallel 50° 35′ N. lat. The Labadie Bank rises between stations 65 and 67.

The intercept on the parallel of latitude 51° 20′ *N*

The axis of the current crossed this parallel slightly to the east of station 83, where it is best characterized by its low salinity and temperature (Fig. 8). The South Wales coastal belt of water shows clearly at station 46.

The continued passage of the coastwise drift from County Cork towards the east is blocked by the Carnsore corner current which contains lighter water. Consequently at the seventh meridian near station 53, the coastal water is forced to turn back as the right-hand component of the Nymphe Bank current. This then and there began to acquire its more composite character. This bottom component of County Cork coastal origin had the properties set out in Table 4.

The intercept on the parallel of latitude 50° 35′ *N.*

There was a 45-mile gap between stations 42 and 88 (Fig. 9), so that the position and degree of sharpness between the mass of Land's End water bathing North Cornwall and the somewhat stagnant water around 6° 10′ W. was not closely established.

The Labadie Bank, lying 80 miles south of Cork Harbour and rising from about 60 fm (110 m) to 34 fm (62 m), is a mere 4 miles wide but 25 miles long. It lies in alignment with the Cockburn Bank.

It is considered that the Labadie and Cockburn Banks may exert a strong influence on currents in the Celtic Sea which have been initiated by other means. If the Labadie Bank has no rock or moraine spine but is a sand bank, it is the consequence and not the cause of this current system. The geophysicists could decide. On this cruise stations 19, 20, 21, 65, 66, 67 were laid out to test this hypothesis. Due to the overcast weather and lack of an echo-sounder on R.V. 'Sabella' at that time, the exact position on top of the bank required for station 66 was not found and so the station had to be omitted.

The Nymphe Bank current (Figs. 7 & 9) cut the parallel 50° 35′ N. at about 7° 50′ W. at station 67 where salinity was minimal (35·13–35·20‰).

The properties of the water at station 65, immediately north-west of the Bank, were markedly different from those of the water composing the Nymphe Bank current running along its south-eastern edge. The high-salinity water could have only come from the western sector of the Celtic Sea.

The water at station 64 had much in common with the water at station 67, but was different from that at the intervening station 65. It is clear that the Labadie Bank held the main Nymphe Bank current on its southeastern flank and that the water at station 65 had a western origin. The history of the water at station 64 will be discussed later (p. 258).

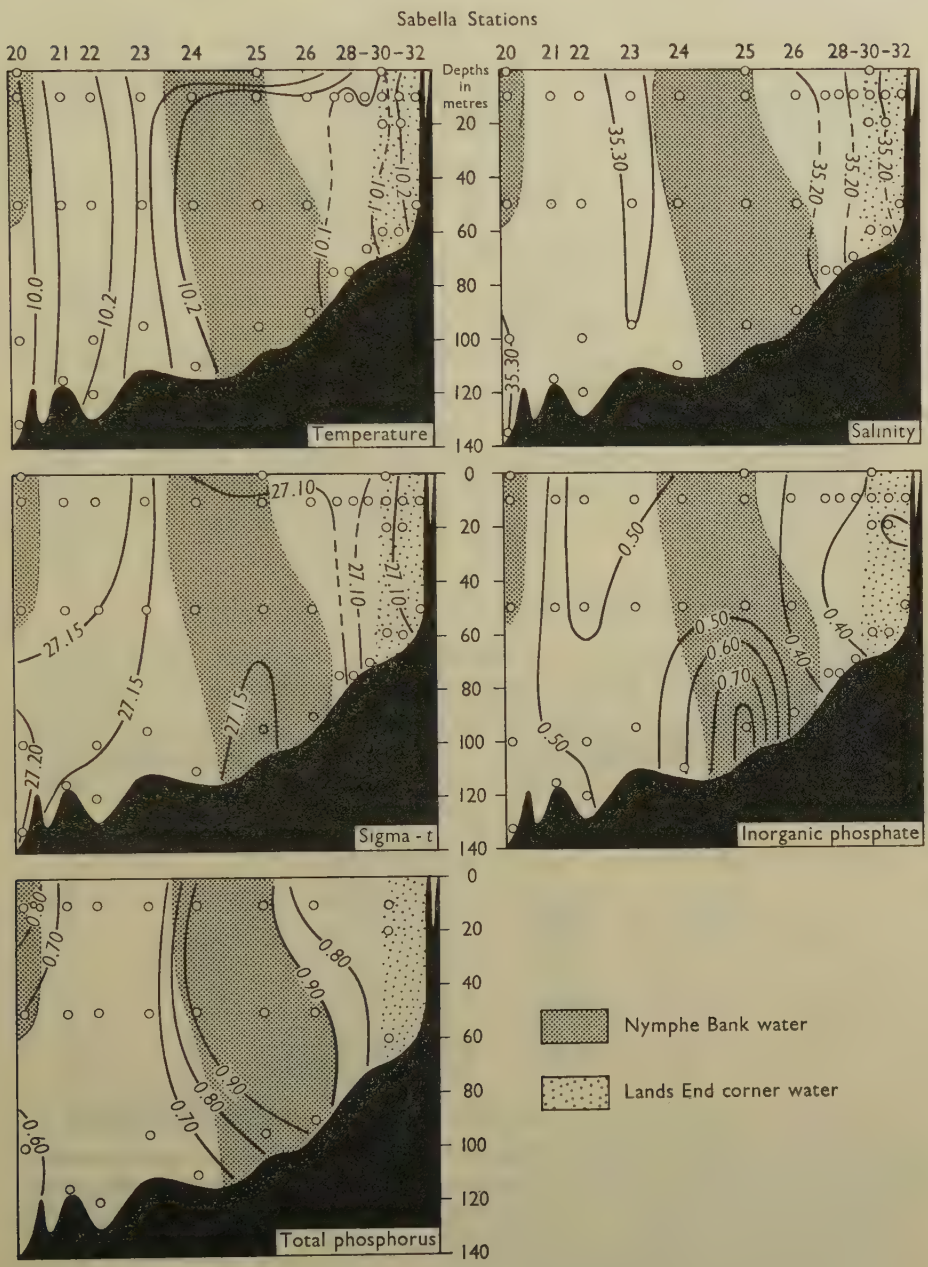

Fig. 10. Section along the parallel 50° N. lat. The Seven Stones Light Vessel lies between stations 29 and 30.

The intercept on the parallel of latitude 50° N.

The most characteristic feature of this section is its lack of character, a quality often noted hereabouts. Even so it is possible to recognize some Nymphe Bank water persisting at stations 24 and 25 (Fig. 10). This continued forward to station 12 (49° 33′ N. lat., 6° 24′ W. long.).

An independent branch of the Nymphe Bank current was present at station 20 and is to be discussed below on this page and p. 265.

The intercept on the meridian 7° 30′ W. long.

The salinity profile (Fig. 11) shows the Carnsore corner water in course of becoming the Nymphe Bank current, present very strongly at station 83 and present at station 82 where it was underpinned by water of higher salinity. To the northward, the current was bordered by the County Cork coastal drift there flowing in the opposite direction. The Nymphe Bank current recurved back across the section at station 24 proceeding south-east. There had been considerable admixture of warmer and more saline water so that the deduction comes from a solid picture of the whole area and not from this section alone.

THE COCKBURN BANK REGION (9° W. LONG.)

Stations 19 and 20 (Fig. 12) were planned close together to test a working hypothesis of the effect the bank may have on currents from or towards the south-west. Accuracy of station position was of much importance, but sights were good hereabouts, so that the error of latitude was unlikely to have exceeded 1 minute of arc and of longitude 2 minutes of arc. The expected sharp boundary between two water masses was found (Table 5).

TABLE 5. PROPERTIES OF THE WATER AT STATIONS 19 AND 20

	Station 19, 49° 55′ N., 9° 05′ W.			Station 20, 49° 57′ N., 9° 06′ W.		
Depth	Temp.	Sal. ‰	σ_t	Temp.	Sal. (‰)	σ_t
0·5	—	—	—	10·00	35·21	27·14
10	10·00	35·32	27·22	9·99	35·21	27·14
50	9·98	35·30	27·21	9·95	35·20	27·14
100	9·98	35·31	27·21	9·96	35·30	27·21
135	9·96	35·28	27·19	9·98	35·30	27·21

At station 20 between the surface and a depth of 50 m lay water similar to that in the Nymphe Bank current and to the water at station 64. Between a depth of 100 m and the bottom at station 20, and at all depths 2 miles away at station 19, lay water 0·1‰ more saline, suggesting water from the west or south but cold, suggesting an origin from the north. The combined indications therefore indicate that this water (at station 19) came from the north-west. A more direct origin from the continental edge west or south-west was also discounted by the low content of phosphate, 0·53 μg atom/l.

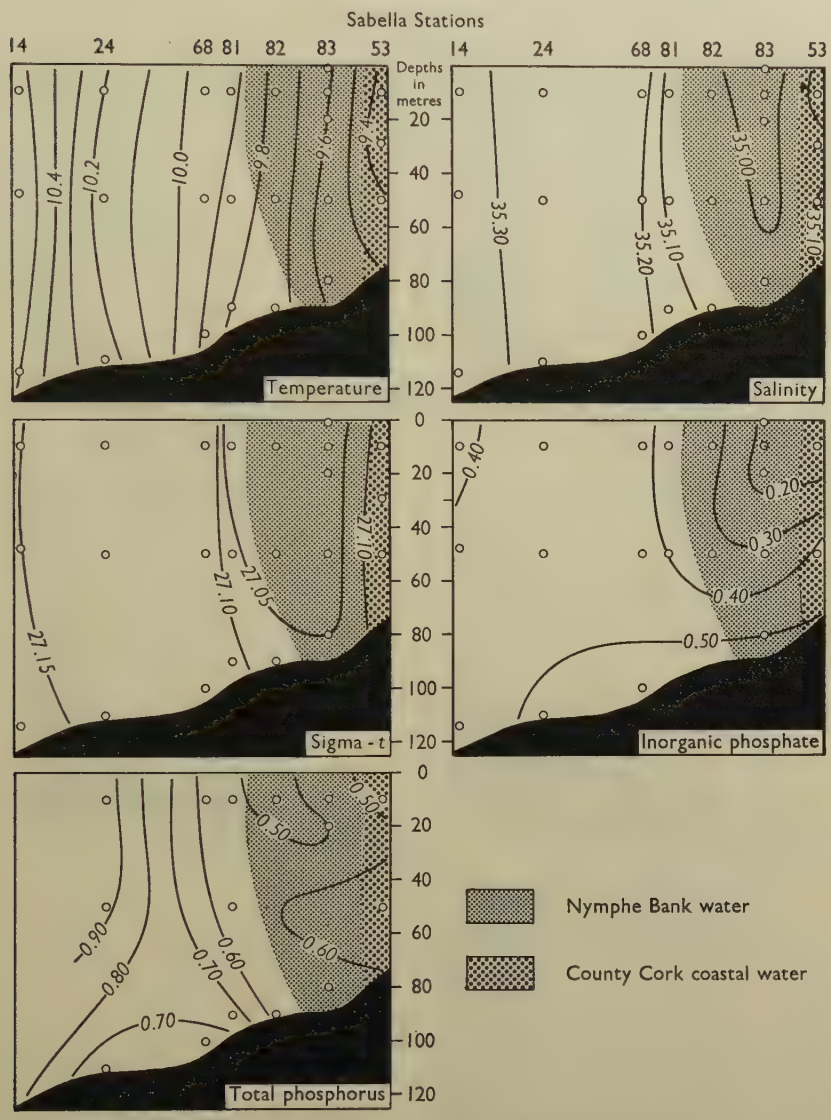

Fig. 11. Section along the meridian 7° 30′ W. long. The axis of the main Nymphe Bank current passed to the west of stations 68 and 81 and after admixture with warmer and more saline water re-cut this section at station 24 travelling south-east. This interpretation has been constructed from the complete solid model rather than from this section alone. Consequently the modified Nymphe Bank water at station 24 is not stippled.

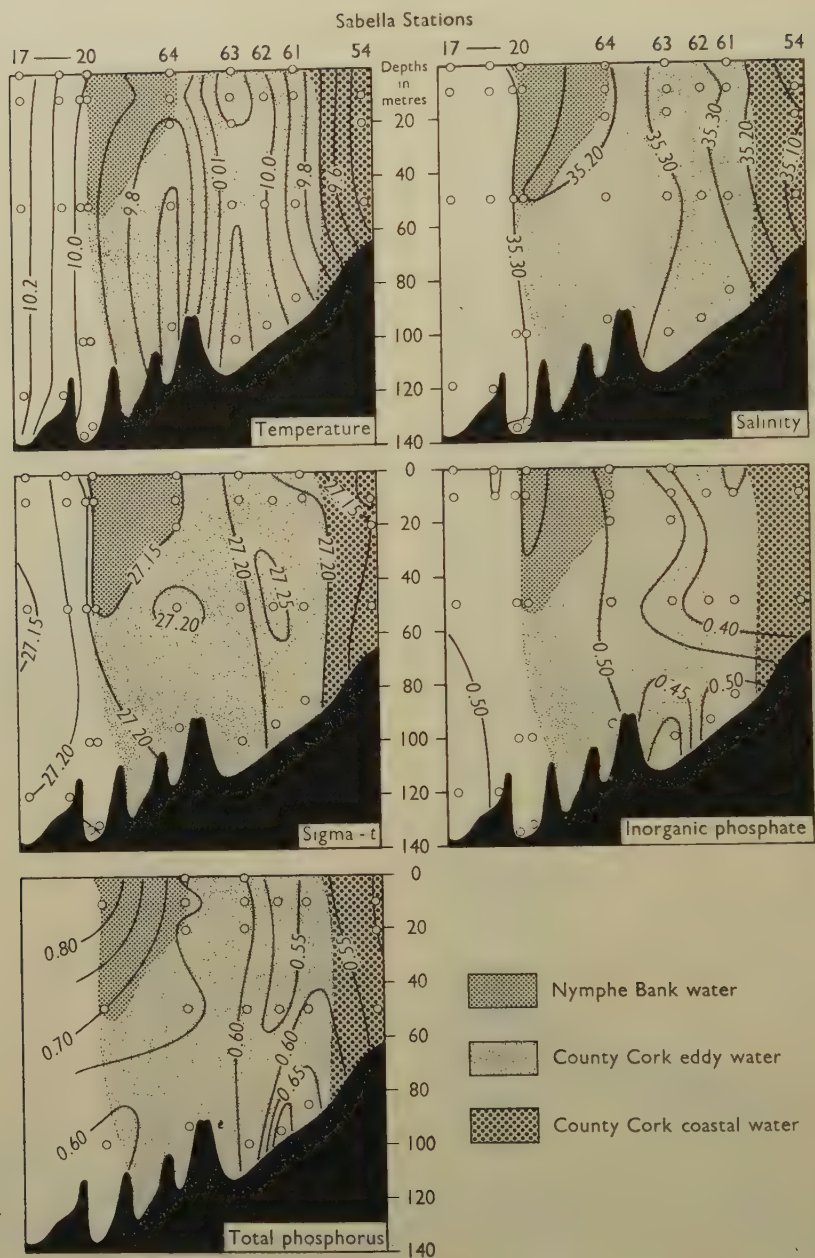

Fig. 12. Section along the meridian 9° W. long. Attention is drawn to the very sharp boundary between stations 19 and 20, 2 miles apart over the divide of the Cockburn Bank.

ON LATITUDE TEMPERATURE DEVIATIONS

Only very late in the investigation was it realized that the deviations from the latitude means of temperature already published (Cooper, 1960*a*, fig. 4 lower, reproduced here as Fig. 13) gave a clear understanding of what had happened. All the water in the central Celtic Sea had been concerned in a 'prevailing drift' from north-west to south-east, but between 24 and 29 March (Cooper, 1960*a*, Table 1) a change of wind had driven a layer of surface water rather more than 50 m thick from the eastern side of the drift towards the western and the break-out had been channelled by bottom topography such as that of the Cockburn Bank.

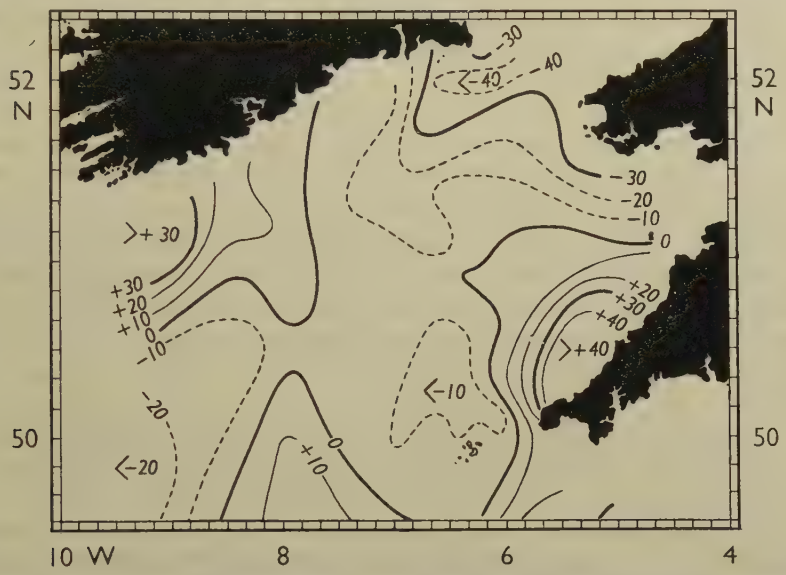

Fig. 13. Deviations of temperatures at 10 m depth from the latitude means (reprinted from Cooper, 1960*a*, fig. 4, lower half). Unit, 0·01° C.

The latitude temperature deviation revealed another component of the drift current which had been overlooked during the working up from the conventional diagrams, i.e. the remains of a warm saline current which had earlier set south from the coast of County Cork between Cape Clear and Kinsale. The northern part of this drift has already been recognized as the stagnating County Cork eddy. The possible correlation with the warm tongue centred on the position 49° 30′ N., 8° 00′ W. had been missed. The water had warmed up during southward transport through 95 minutes of arc by about 0·45°, instead of 0·67° computed from the coefficient established for the whole area.

The latitude temperature diagram provides strong evidence to suggest that these two separated water masses were indeed the same water mass which had been severed by a flow across it of water from the Nymphe Bank.

This would mean that the diagram (Fig. 13) portrays a level crossing in the sea at about 50° 30′ N., 8° W., with water movements taking turns as do trains and cars on a railway level crossing. First, there had been a south-south-easterly composite drift setting away from the Irish coast along its whole length from Cape Clear to Carnsore Point. The eastern component of this drift had consisted of cold Nymphe Bank water and the western of warmer and more saline water from between Cape Clear and Kinsale. Later a considerable arm of the Nymphe Bank component pushed out to the south-west cutting across the Cape Clear component to reach at least as far as the Cockburn Bank. At the time the cruise was run this second movement had passed its peak and was being nipped by a fresh south-south-easterly extension of County Cork 'eddy' water.

Previously (Cooper, 1960*b*, fig. 16 and table 2) a possible south-easterly bottom flow has been traced from 'Sabella' station 16 on 19 April towards 'Discovery' station 2654 on 11 May. Now that a probable identity in water type has been established between 'Sabella' stations 14 and 15 and stations 62 and 63, it is not unreasonable to assume that water similar to station 16 would have been found west of our area of operations in about 51° N. 9° 30′ W. At any rate there is now no need to seek an origin of the bottom water at station 16 to the west or south-west as was earlier suggested (Cooper, 1960*b*, p. 197) by a process of exclusion.

THE PHOSPHATE BALANCE SHEET OF THE NYMPHE BANK CURRENT

We have seen that one component of the drift when it had passed Carnsore Point was poor in phosphorus (inorganic phosphate 0·45, total phosphorus 0·61 μg-atom/l.). Over the muddy bottoms south of Ireland, this water was enriched by decomposition of organic material (Cooper, 1951). The rich water up-welled mostly off Waterford due to winds channelled by the land topography (Fig. 6, p. 247). The distribution of phosphate in the Nymphe Bank current therefore became streaky. Similar up-welling could contribute to an explanation of the high fisheries productivity observed hereabouts by the Lowestoft Laboratory in 1958–9.

In April 1950, by the time the current had reached station 83, 5000 Harvey units of plant pigments had been produced, inorganic phosphate had sunk to 0·17 μg-atom/l. while the organic phosphorus fraction rose to 0·36 μg-atom/l.

At other stations (e.g. station 81, cf. Cooper, 1960*c*, table 5) almost all the high total phosphorus content in bottom water was present in the inorganic

form. In 1950 I attributed some of these results to errors of sampling or analysis. But now, in the light of the work of Strickland & Austin (1960) on the cycle of phosphorus in the North Pacific, I am inclined to accept and interpret them in terms of my experiments (Cooper, 1935) on the regeneration of phosphate from animal plankton. On this view, disturbance of the muddy veneer yielded to the water not only suspended matter containing organically bound phosphorus but also micro-organisms able to hydrolyse it. These organisms carried the process further by hydrolysing *dissolved* organic compounds which were elsewhere resistant to attack.

Much is implicit in this hypothesis since similar organisms may not only hydrolyse bound phosphorus but may chemically mature a water in subtler ways (Lucas, 1955). Combined with an effective means of up-welling, we would have a working hypothesis of great power. It is the more necessary, therefore, to provide it with a basis stronger than a few analyses which had first been dismissed as suspect.

ENRICHMENT OF SURFACE WATER WEST OF SCILLY

High contents of total phosphorus were found at 10 and 50 m depth at stations 20, 24, 25, 26 on April 20, 1950 (0·85–0·96 μg-atom/l.). The excess over the average for the cruise was due to organically bound phosphorus. The following possible explanations have been pursued and abandoned as untenable: (1) submarine eagres from canyons in the continental slope to the south and south-west; (2) vortex up-welling (Cooper, 1960*a*), i.e., up-welling along the lane followed by a cyclonic depression which passed over the Celtic Sea on 17 April[1]; (3) hydrodynamic up-welling.

No explanation for the high upper water content of organic phosphorus can be offered in terms of any of these. The four determinations, for inorganic and for total phosphorus at stations 25 and 26, when considered in pairs, are inconsistent. There remains the possibility that the high total phosphorus content originated from the muddy area 50 miles N.N.E. (Cooper, 1951) and after transport by the bottom current up-welled in the rising centre associated with the Scilly Isles (Cooper, 1960*a*). Though the basic data are scanty, we have had other rich catches near station 25. One such was diagnostic of the '*elegans*-type' water we sampled for Dr D. P. Wilson (Wilson, 1951, expts. 1 and 2). The possibility of rich water arriving as a bottom current from the north and being brought up in a rising centre deserves further study.

[1] The course of this depression was wrong by 50 miles and it was not strong enough to be effective. I am indebted to Dr R. C. Sutcliffe of the Meteorological Office for a critical discussion of the synoptic situation.

NUTRIENTS AND PHYTOPLANKTON

The nutrient contents of the several types of water which have been recognized are set out in Table 6. A primary objective of the cruise was to find where the rich water, present in the English Channel in the 1920's, came from. For this purpose the cruise was a complete failure since over the Celtic Sea as a whole, the phosphate content was too low.

TABLE 6. THE DISTRIBUTION OF NUTRIENTS IN APRIL 1950

(μg-atom/l.)

Water type	Total phosphorus	Inorganic phosphate	Silicate
English Channel south of Cornwall	0·66	0·44	—
Land's End corner current	0·57	0·47	3·3
North Cornwall (English Channel origin)	0·57	0·44	—
South Wales coastal	0·78	0·60	5·2
Carnsore corner current	0·61	0·31–0·45	2·4
Waterford up-welling	0·69	0·61	—
County Cork coastal	0·57	0·36	—
Nymphe Bank current	Very variable		—
Bristol Channel bottom water	0·61	0·45	—
Cockburn Bank area	0·71	0·45	—
Bottom water over mud	0·71	0·55	—
West Scillies enriched water	0·84	0·50	—

In 1950 we were examining the possibility that total phosphorus might be more nearly conservative than inorganic phosphate. This has proved not to be so. During the spring outburst of phytoplankton and the cycle of animal growth which develops from it, the total phosphorus content commonly decreases much as phosphate. The loss has gone into material which escapes the water-bottle. Again, much of the total phosphorus becomes particulate, and its distribution therefore tends to resemble that of iron (Cooper, 1948; but see Armstrong 1957). The analyst may correctly return a high figure for his sample but this may not truly represent the water from which it was drawn. If total phosphorus determinations are to be dependable they need to be replicated. Again, in April 1950 one set of bottles used for storage of total phosphorus samples gave off phosphate and the spurious results with them had to be rejected.

The results for bottom samples which seem analytically sound are presented here (Fig. 14), (*a*) as first drawn, and (*b*) adjusted to accord with the probable movements of the bottom water in the Celtic Sea. This adjustment has involved constructing a course for water containing over 0·7 μg-atom/l. total phosphorus from north to south along the meridian 6° 45′ W. between the grid stations. This is by no means impossible and is in line with the bolus concept in course of development for bottom water movements (Cooper, 1960*b*). Good reasons were found for rejecting alternative explanations.

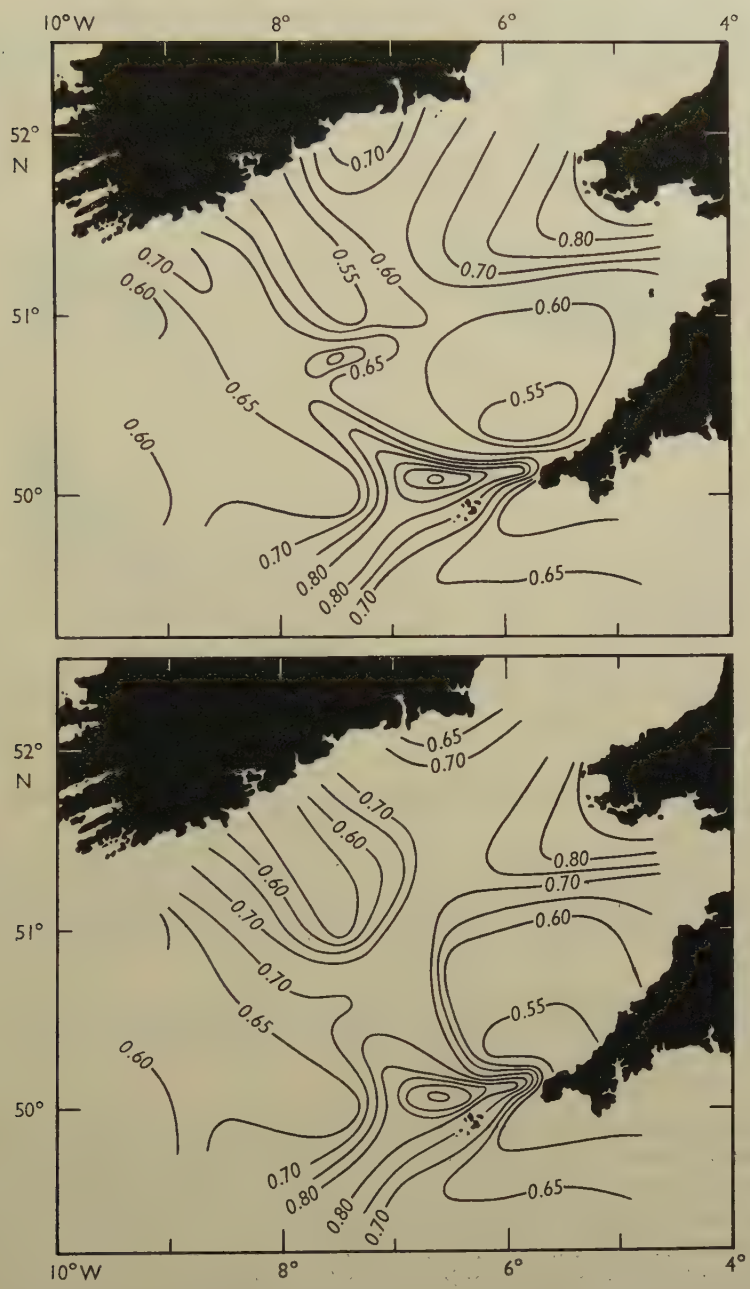

Fig. 14. Content of total phosphorus (μg-atom/l.) in bottom water as first contoured (upper) and adjusted to accord with the probable movements of bottom waters in March–April 1950. For station positions see Fig. 1, except that no reliable observations are available at stations 14–19.

THE PLANKTON CONTENT OF WATER MASSES

At a number of stations the Harvey quantitative net was lowered to within a metre or so of the bottom and then drawn back to the surface. (Damage to the net prevented its use at station 47–54 until it could be repaired in Queenstown Harbour.) The plant pigments in the plankton catch (Fig. 15) were assessed in terms of Harvey units (Harvey, 1934).

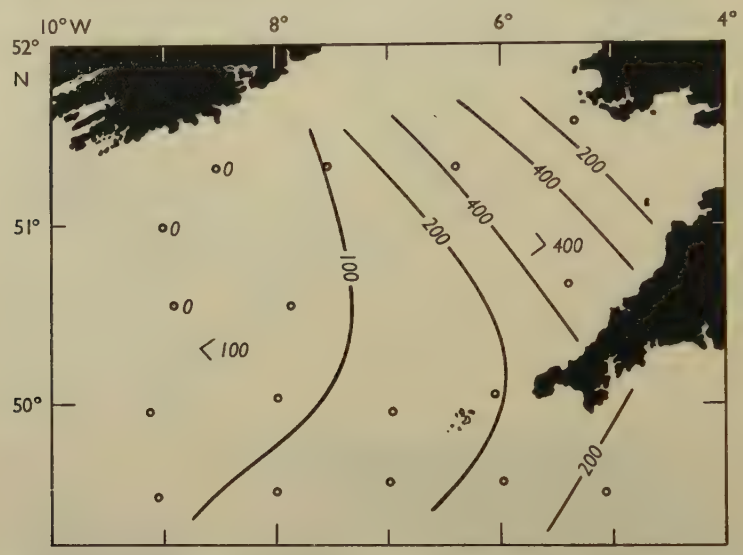

Fig. 15. The distribution of plant pigments as Harvey units per cubic metre of water column.

It was intended to work the 1 m net for zooplankton and this was done at stations 9, 11, 15, 17 and 20. Loss of the nets, however, forced us to use standard oblique hauls of the 2 m stramin net (YFT) at the remaining stations sampled.

Phytoplankton was reasonably abundant in the English Channel and the Celtic Sea south of the Scilly Isles and in the waters derived from it around Land's End and north of Cornwall. In the County Cork eddy water plant pigments were undetectable.

The zooplankton even allowing for the fact that the 2 m stramin net fails to catch a great many small forms, was extremely sparse. There were no indicators of south-western or of oceanic waters. *Sagitta elegans* occurred in small number in some samples in all the water masses recognized. *S. setosa* was present in all the samples from the South Wales coastal water but, absent from water in the Nymphe Bank current (except for very few at station 85) and the County Cork eddy water. *Tomopteris* was found only in the South Wales coastal water and off Land's End. *Saggita serratodentata*, an indicator

of water from the continental slope, occurred in all the waters recognized as County Cork eddy water and only there.

Pilchard eggs were abundant only in the Land's End corner current and a few were found at stations 42, 67 and 88.

EVENTS WEST OF THE MERIDIAN 9° W.

The conclusions as to the drift current flowing in the northern Celtic Sea between 7° and 9° west prompt the question as to what may have been happening still farther west. On 19 March 1955 R.V. 'Sarsia' worked a section 40 miles long up the continental slope along the parallel 50° 34′ N. between 11° 28′ W. and 10° 20′ W. (Fig. 16). The iso-lines of all properties except salinity sloped steeply upwards towards the slope. The isopycnals showed that

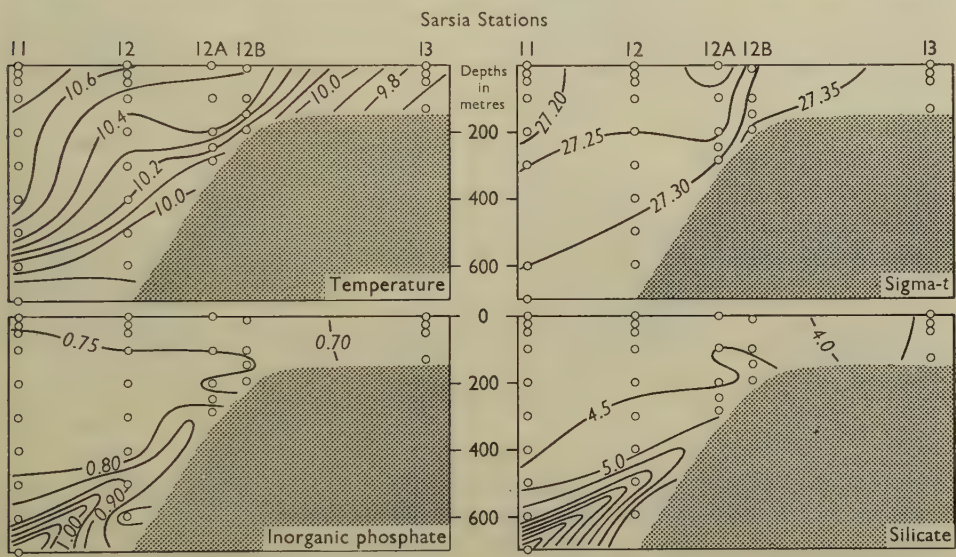

Fig. 16. East–west section over the continental slope on 19 March 1955 along the parallel 50° 34′ N. Salinity was almost uniform (35·42–35·47‰) except at station 12B (35·53‰). Silicate and phosphate are reported as μg-atom/l.

a current was running south above the break of the slope and extended down the slope to a depth of 600 m or more. The nutrients betrayed a similar slope indicating moderate up-welling.

We now have strong evidence for a south-going current in 7° W. and in 10° 50′ W. in March–April and an inference that it may run intermittently in 9° W. It is not unreasonable to conclude that everywhere between 50° and 51° N. lat. and between 7° and 11° W. long. there is in these months a general drift of water from north to south or south-east.

Also on 18 March 1955 there was a less strongly developed current setting south-east over the slope south of the Celtic Sea in about 7° 40′ W. (Fig. 17). This weak current is in line with evidence that has already been presented for that area (Cooper, 1960*b*). i.e. the broad current after it has lost the component which turns east into the English Channel, weakens and turns into alignment with the slope of the southern Celtic Sea.

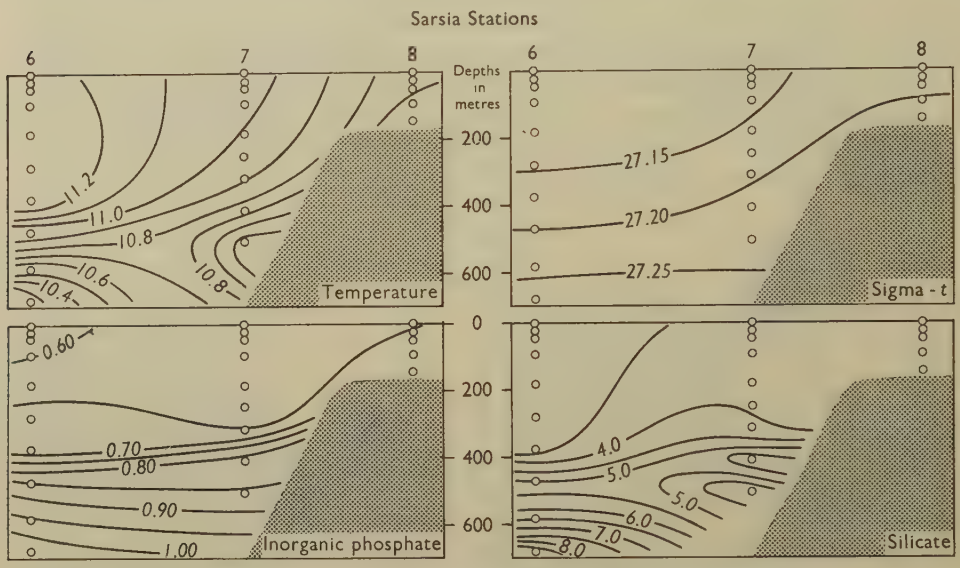

Fig. 17. Approximately north–south section over the continental slope in about 7° 40′ W. long.

THE GENERALIZED 'PREVAILING DRIFT CURRENT' AND THE EDDY SYSTEM IN THE NORTH-EAST

The cyclonic circulation of the north-eastern Celtic Sea first suggested by Matthews (1914) has been established for the early spring of 1950 (Fig. 18). The entry of warm, saline water intermittently in the Land's End corner current to the waters north of Cornwall may be said to initiate the circulation. In the shallow Bristol Channel this water is strongly cooled and diluted by run-off to give the cold but light South Wales coastal current flowing west. This current becomes enriched in phosphate from sewage but it has not been shown that this enrichment was followed by extensive production of plants and animals.

In St George's Channel there was a component of movement from east to west, but if there was any direct transfer of water it was through an area centred on 51° 45′ N., 6° 15′ W. which we did not sample. The South Wales water rich in phosphate and silicate turned St David's Head into Cardigan

Bay and was replaced mainly by a nutrient-poor water with similar temperature, salinity and density flowing south along the coast of County Wexford around Carnsore Point. The component of movement from east to west was therefore at best a resolvent from a diffuse cyclonic movement which involved much of the Irish Sea.

The Nymphe Bank is an extensive area with a depth of about 70 m lying south of County Waterford and County Wexford. Though this depth is considerable, even so the rate of fall of temperature over it in cold weather will be greater than in the vertically homogenous deeper water to the south. The Nymphe Bank is therefore an area where in winter cold water may become relatively colder still. The loop in the iso-lines in Fig. 13 (p. 257) centred on 51° 30′ N. 6° 30′ W. is the result of this property of the Nymphe Bank in winter.

This cold water escaped to the southward to provide a large statistically stagnant water mass centred on a position about 51 °N., 6° 45′ W. Probably its most usual movement is as a wind drift constrained by the topography of the land masses towards the Scilly Islands. The course in early April 1950 may have been as in the section shown in Fig. 7. (p. 249) and in Fig. 18. The cold pocket with a latitude deviation less than −10 (i.e. a temperature 0·1° C colder than the latitude mean) lying north of Scilly, may provide the water which Carruthers, Lawford & Veley (1951) so frequently find moving past the Seven Stones Light Vessel in a direction between east and south-east.

An alternative movement towards the south-west had also taken place not long before the April cruise. Channelled in direction by the Cockburn Bank, it was still running as a current with a sharp boundary between stations 19 and 20.

Piecing together all the arguments presented for March, April and May 1950 and in March 1955 in these several papers, we have a picture of a broad current in March–April setting south-east or south-south-east from the south coast of Ireland across the Celtic Sea towards the mouth of the English Channel and Brittany (Fig. 18).

This was recruited in some measure from water coming down the east coast of Ireland and probably by a large body of saline water recruited from the ocean south-west of County Kerry. A rather qualitative assessment would suggest, in spite of what has been written of St George's Channel, that a considerable volume of cold water of intermediate salinity (say 35·1‰) must be supplied from the east through the position 51° 45′ N., 6° 15′ W. where we had no observations.

During April and May water was drawn off in an easterly direction or somewhat south of east into the English Channel. On this view the cyclonic eddy of the north-eastern Celtic Sea in early spring is a secondary consequence of this extensive wide wind drift and not a primary event.

Some time before the April cruise there had been a break-out of Nymphe Bank water flowing on the eastern side of the stream across to the western side, cutting in two the more saline and warmer water mass composing it.

Current charts (e.g. Admiralty Hydrographic Department, 1954, p. 7) show a drift between east and south-east. Such charts, derived from the drift of ships, represent only the top 4 m or so of the sea. The present study in April 1950 has indicated in the Northern Celtic Sea a drift about 45° to the right of this direction, a deviation to be expected when one studies movement of the whole water column rather than the top 4 m.

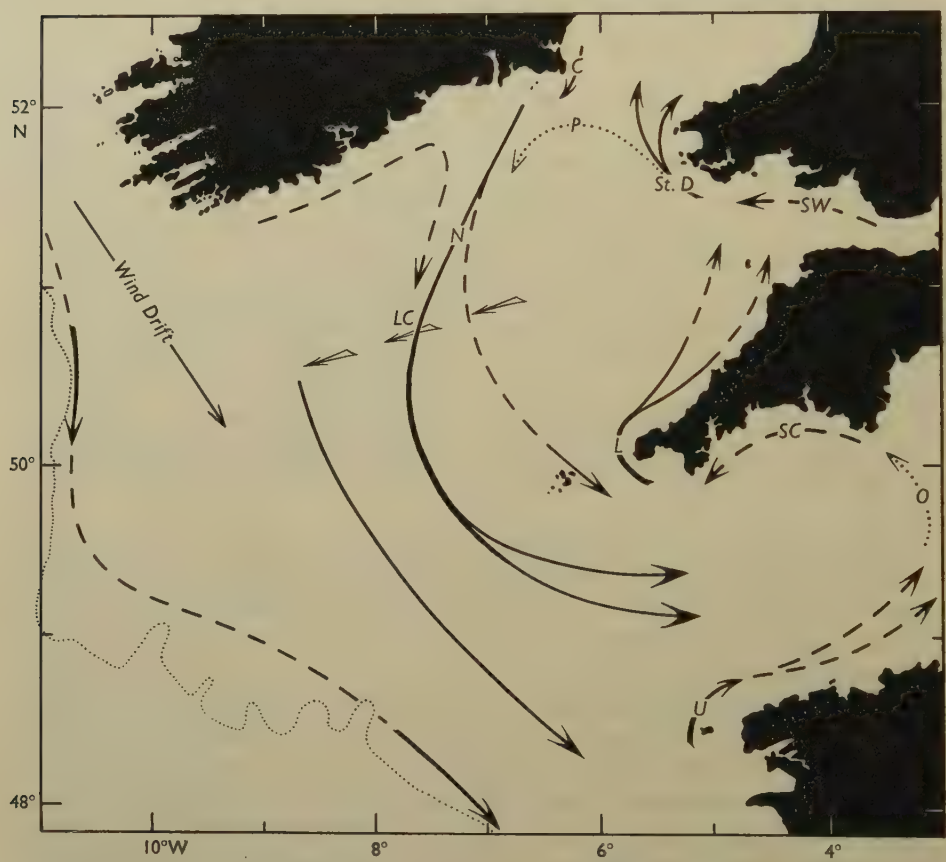

Fig. 18. The pattern of currents in the Celtic Sea in April 1950. For an explanation of code letters see pp. 267–8.

Evidence has already been adduced that recruitment of new water to North Cornwall occurs periodically during neap tides. Somewhere there must be a compensating pulse to enable an equal volume of water to escape from the

north-eastern eddy. It is tempting to believe that the cold water observed on the ninth meridian between 50° and 50° 35′ N. may have been a consequence of this compensation pulse.

Acknowledgement of the generous help received was made earlier (Cooper, 1960*c*, p. 657). Further, I am much indebted to Mr P. G. Corbin for the zooplankton distribution pp. 262–3 and to Mr F. A. J. Armstrong for the analyses of compounds of phosphorus and silicon.

THE WAY AHEAD IN THE CELTIC SEA

The preparation of the papers on the Celtic Sea in this and the two previous issues of this *Journal* has involved much work spread over 10 years. For the years in question much valuable factual information about the area has been produced and some suggestions for further work have emerged. But it must be admitted that the main conclusions are no more than (*a*) that prevailing winds produce prevailing currents—an axiom, and (*b*) that the conclusions on circulation drawn by Matthews (1914) and by Russell (1935*a*, *b*) using biological indicators are confirmed. Such an inadequate return from so much effort gives cause to pause to consider the way ahead. The model of our 1950 programme is not the one to follow. The most fertile lines of work, to my mind, are (*a*) punctilious investigations in crucial regions such as major headlands and experimental sites such as station E 1, (*b*) global studies such as those attempting to relate fishery success with global events like fluctuations in polar climates, and (*c*) studies of the chemical factors which control the success or failure of indicator species and commercial fish.

SUMMARY OF THE 'PREVAILING' CURRENT SYSTEM IN SPRING

The pattern of currents deduced largely on the basis of work in 1950 and described in this and the preceding papers is summarized in Fig. 18 (p. 266). Features of special interest are lettered:

U Ushant corner current. Favoured by very wet weather over western France and by very mild weather over the sea. May be completely suppressed during dry or cold weather.

O Occasional drift, consequent on very strong development of the Ushant corner current or upon more water being pressed into the western English Channel than the Straits of Dover can pass to the North Sea.

SC South Cornwall coastal current, which may be a compensation current to get rid of excess water from the western English Channel. It may be assisted by heavy rainfall or very mild weather over south-western England.

L Land's End corner current, running most strongly at neap tides and favoured by very wet weather over south Devon and Cornwall and by mild weather. May be suppressed at spring tides and/or during cold and dry weather.

SW	South Wales coastal current, dependent on the amount of drainage from the Severn and other considerable rivers draining into the Bristol Channel. Deductions based very largely on inference, more observations needed.
St D	St David's corner current, likely to share the characters of the Land's End Corner current.
C	Carnsore corner current probably intermittent and small compared with the corner currents around Ushant, Land's End and St David's Head.
N	The Nymphe Bank current.
LC	'Level crossing', see p. 258.
P	Possible drift in an area where there were no stations.

This pattern of currents in spring is likely to flow when winds prevail from the west or south-west. Prolonged winds from other quarters may distort it, indeed prolonged easterly winds may destroy it completely.

Again, the pattern of currents in Fig. 18 has no application in the months June–September.

SUMMARY

The circulation of the northern and north-eastern Celtic Sea, has been studied, using especially observations made in April 1950.

The Celtic Sea in that month was 0·5° C warmer than the average temperature 20 years earlier.

The following distinctive water masses have been recognized (i) Land's End water; (ii) South Wales coastal water; (iii) Carnsore corner current water; (iv) Nymphe Bank water, including a component enriched by up-welling off Waterford; (v) County Cork eddy water.

The South Wales coastal water, though rich in nutrients presumably derived from sewage effluents, was not very productive. A parallel is drawn with the Hyperion sewage scheme of the City of Los Angeles.

The low-salinity water present in the centre of St George's Channel in summer is attributed to flow from the coast of South Wales. In the autumn water is recruited more directly from the Celtic Sea.

The silicate content of the Carnsore corner current was half that in South Wales coastal water on the opposite side of the Channel.

Up-welling of water enriched by breakdown of bottom muds occurred off Waterford.

The drift system in the central Celtic Sea is dominated by the prevailing winds. The drift in April 1950 was a composite affair and it has been possible to dissect some of the components and to show how they interplay.

In about 7° W. long., a mixed water mass sets south from the Nymphe Bank and was identified for more than 100 miles.

A tentative explanation is offered for the distribution of compounds of phosphorus in terms of regeneration from bottom deposits. The distribution of plant pigments and of some animals was related to the history of the water masses.

In March 1955 sections over the continental slope west and south of the Celtic Sea enable a pattern of wind-drift currents to be established (Fig. 18, p. 265). The drift of plankton is discussed.

Appendix added in proof

Since the paper was written Southward (1961) has published an article on the distribution of plankton animals in the English Channel and Western Approaches in 1955 and 1957. His conclusions are in close accord with those presented here. Though there are differences in detail, as might be expected when comparing different years, the broad agreement is striking. My contention that biologists may derive a sufficient understanding of water movements by study of their catches only is strongly reinforced.

I agree with his conclusions, at least in spring, that Russell's 'Western' water may be better described as 'North Western' water. As spring gives way to summer, experience may show that the term 'Western' remains more appropriate.

It is improbable that the high phosphate observed at Station E 1 early in 1955 was connected with the high phosphate bathing the upper continental slope south-west of Ireland. Though not yet examined in detail it is more probable that, as in 1950, the high phosphate content at E 1 had been earlier created by gales disturbing the muddy deposits in Lyme Bay (Cooper, 1958), followed by a coastwise drift to the west.

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THE TAPETUM IN *SCYLIORHINUS CANICULA*

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(Text-figs. 1–3)

Elasmobranchs are peculiar in having a chorioidal tapetum which, according to Franz (1905), is occlusible. The tapetum lies in the outer region of the chorioid and contains flat cells, in which there is a reflecting material believed to be guanine. Over much of the eye they are arranged obliquely to the retinal surface, extending outwards to the choriocapillaris like overlapping tiles. A flattened nucleus lies midway along the external surface of each plate. The inner surface of the tapetum is occupied by a single layer of spherical pigment cells possessing processes which may extend outwards over the reflecting plates or cells. These pigment cells contain a dark pigment, presumably melanin. When the pigment cell processes extend all the way to the choriocapillaris and cover the reflecting plates, the retina appears black, the tapetum is occluded and no light is reflected back upon the retina. This description is the one usually presented for the elasmobranch eye (Franz, 1913, 1934; Walls, 1942, Rochon-Duvigneaud, 1958; Nicol, 1960).

A pigment cell usually has one process; occasionally, it possesses two processes, supplying two reflecting or tapetal plates. It appears from Franz's accounts that the processes of the pigment cells move outwards and inwards, in contrast to the behaviour of skin melanophores, which have fixed processes in which pigment granules make excursions. The chorioid, internal to the layer of migratory pigment cells, has flattened static pigment cells containing black pigment.

Some special features of the elasmobranch tapetum are these. A stripe ('tapetal stripe, horizontal stripe') across the fundus appears especially bright. The stripe lies slightly above the optic papilla and is usually horizontal, but slightly oblique in *Scyliorhinus*. In this region the tapetal plates are undulating and tangential to the retinal surface, and the tapetum is exposed, fixed and non-occlusible. Often the ventral region of the eye is black: this, the ventral field, is triangular in shape, the apex extending up to, and sometimes including, the optic papilla (*Scyliorhinus*, *Raja*). The pigment cell processes are closely spaced and, together with the reflecting plates, are arranged vertically. In the ventral field the tapetum is permanently black and occluded. Sometimes there is a permanent black band at the top of the fundus, as in *Scyliorhinus* (see Franz, 1931, 1934). A second type of tapetum

occurs in *Lamna cornubica*, in which the tapetal plates are superposed in layers parallel to the surface of the retina. Melanophores and their processes extend through the entire tapetum (Rochon-Duvigneaud, 1943). A tapetum lucidum is absent in *Laemargus*, *Myliobatis* and *Cetorhinus maximus* (see Franz, 1934).

It is not obvious from published descriptions what regions of the elasmobranch tapetum are occlusible. The tapetal stripe across the fundus is non-occlusible and permanently bright, the ventral and dorsal fields occluded and permanently black. Presumably, lateral fields and those tapetal areas of the fundus lying between the central tapetal or horizontal stripe and the ventral and dorsal black fields are occlusible.

The present study was undertaken to determine factors and agencies controlling pigment migration in the tapetum, especially direct and indirect influence of light, possibility of diurnal rhythmicity, and existence of neural or endocrine control.

MATERIALS AND METHODS

Dogfish, *Scyliorhinus canicula*, were kept in tanks at 15° C and were treated in ways to be described. In each test three specimens were used, unless stated otherwise.

Series 1. Dogfish were placed in the dark at 17.00 h (approx.), and they were sacrificed in the dark next day at 06.00, 12.00, 18.00 and 24.00 h (13, 19, 25 and 31 h in the dark, respectively).

Series 2. Dogfish were placed in the dark at 17.00 h (approx.), light came on at 06.00 hr next day, and fish were sacrificed in the light at 12.00, 18.00, 24.00 h that day, and 06.00 h next day (6, 12, 18 and 24 h in the light, respectively). Lights were two 100 W bulbs.

Series 3. Dogfish were placed in the dark at 17.00 h (approx.). Fish were killed next morning, in the dark, at 09.30 h, the eyes were excised and the lenses removed. Eyes were then placed in Nicholls's selachian Ringer at 15° C, and kept in darkness or light for periods of 1 and 2 h. Lights were two 100 W bulbs.

Series 4. Dogfish were placed in a tank which was continuously illuminated from below by two 75 W bulbs; other light was excluded. One fish was killed at each of these periods: 4, 9, 18 and 38 days.

Series 5. Two dogfish, in the dark overnight, were killed in the morning. Eyes were removed, opened, and placed in Ringer; one eye from each fish was kept in the dark for an hour, the other exposed to daylight for an hour, when the two eyes were compared.

Series 6. Two dogfish were placed in the dark for 30 min, then subjected to bright light (two 500 W photofloods) for 2½ h. Eyes of these fish were dissected out in the light and examined.

Eyes of dogfish which were killed in the dark were dissected out under a

dim red light. When these eyes were preserved, they were placed in fixative in the dark. Dogfish exposed to light were killed in the light, and their eyes dissected out and preserved in the light. Eyes were fixed in Zenker's fluid; the fixed eyes were kept in the dark or light for an hour or so according to their previous history.

For microscopic examination eyes were embedded in polyester wax and sectioned horizontally at 10 μ. To estimate the degree of pigment dispersion, an index of 1 to 5 was used: 1, processes fully retracted; 2, one-quarter expanded; 3, half expanded; 4, three-quarters expanded; 5, fully expanded. Every 100th section was examined. Pigment cells were examined in the centre and the extreme anterior and posterior levels of the eye in each section, and the position of the pigment processes was noted.

RESULTS

A dogfish eye, taken from an animal which had been in the dark, has a black ventral field and a black dorsal band. Much of the fundus, apart from the black areas, is cream-coloured, a rose-coloured band crosses the centre of the fundus, and the regions adjacent to the black areas show a blue-green tint. When an excised opened eye is exposed to light, the rose-colour soon fades and the back and sides of the eye become blue-green, except for the fixed ventral field and dorsal black band.

Apart from the fading of the rose tint across the central fundus, no difference was observed between the eyes of fish kept in the dark and in the light. The appearance, observed in sections, of pigment cells at comparable levels in the tapeta of fish kept in darkness and in light was the same. The typical pattern is shown in Table 1, from the eye of a dogfish kept in the light. For comparison, measurements are given for a fish kept in the dark for an equal period (Table 2). Strong light (sunlight) did not cause the light areas of the excised eye to darken. Prolonged exposure (up to 38 days) to ventral illumination produced no alteration in the configuration of light and dark areas in the dogfish eye.

The retina, dissected from the eye of a dark-adapted fish, showed a slight purple tint, owing to the presence of visual purple. In a dissected piece of retina, the coloration appeared greater in the region of the horizontal stripe than in the ventral field. On exposing the dissected retina to light the rose colour soon faded. The rods in the region of the horizontal stripe are about twice as long as elsewhere, and to this circumstance may be ascribed the deeper coloration observed there.

In the tapetal stripe, above the optic papilla, the tapetal plates are very long and extend towards the surface at very acute angles; the bases of the plates are nearly or virtually parallel with the base of the retina (Fig. 1). In the anterior half of the fundus, the plates slope posteriorly; in the posterior half,

TABLE 1. PIGMENT CELL PATTERN FROM FISH KEPT IN LIGHT

Fish kept in the light for 18 h (13 h in the dark followed by 18 h in the light).

Level dorso-ventrally Section	Pigment cell index		
	Anterior margin	Centre of fundus	Posterior margin
100	5	—	4
200	4	—	4
300	4	1	3
400	2	1	3
500	2	1	3
600	2	1	1
700	2	1	2
800	4	1	3
900	4	1	3
950 optic nerve			
1000	5	1	4
1100	5	5	4

TABLE 2. PIGMENT CELL PATTERN FROM FISH KEPT IN DARK

Fish kept in the dark for 13 + 18 h (total of 31 h in darkness).

Level dorso-ventrally Section	Pigment cell index		
	Anterior margin	Centre of fundus	Posterior margin
100	5	4	5
200	4	1	3
300	3	1	2
400	1	1	3
500	1	1	2
575	1	1	2
650	2	1	2
700	3	2	3
optic nerve			
800	4	5	3
900	5	5	4
1000	5	5	5

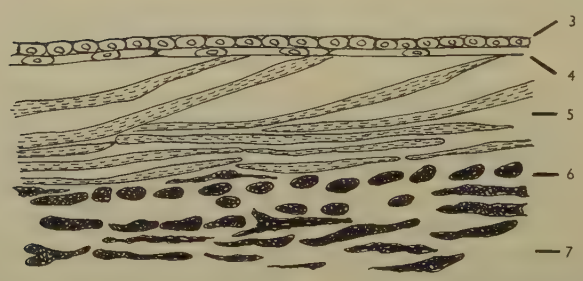

Fig. 1. This and subsequent figures are of sections through the chorioid and contiguous retina of *Scyliorhinus canicula*. × 543. Fundus, region of horizontal tapetal stripe, superior to optic papilla. Pigment cell processes wholly basal (index 1). Longitudinal horizontal section. 1, outer nuclear layer. 2, rods. 3, pigment epithelium (without pigment). 4, choriocapillaris. 5, tapetal layer, 6, pigment cells with processes (layer of migratory cells). 7, unmodified chorioid, containing static flat pigment cells. 8, scleral cartilage.

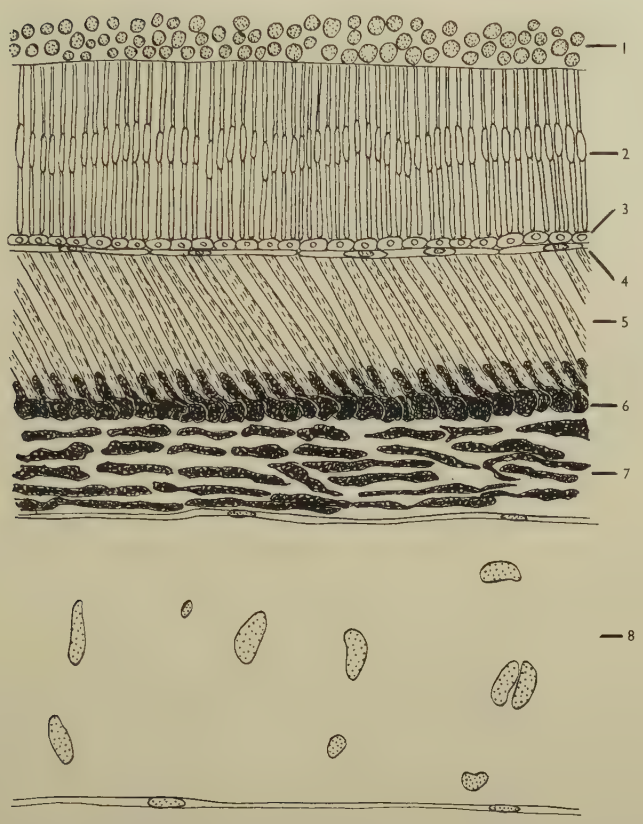

Fig. 2. Longitudinal horizontal section, anterior region, superior to optic papilla. Processes of pigment cells partially extended (index 2). Numbering as in Fig. 1.

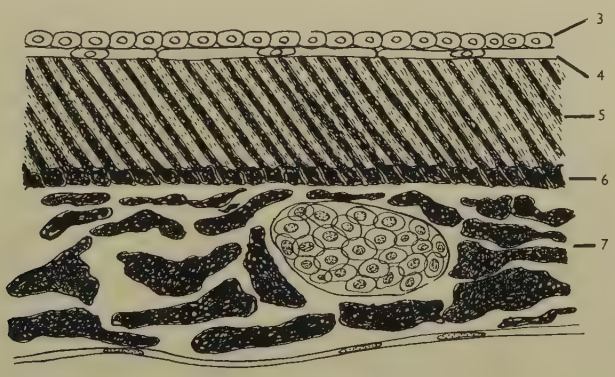

Fig. 3. Vertical transverse section through ventral field. Pigment processes extended (index 5).

anteriorly. In anterior and posterior quadrants of the eye, the tapetal plates are short and are aligned almost vertically to the choriocapillaris. The degree of dispersion of black pigment along the tapetal plates varies with position (see Tables 1 and 2, Fig. 2). In the dorsal and ventral pigmented fields the processes of the pigment cells are fully extended and reach the outer surface of the tapetum (Fig. 3).

Sections from an eye fixed in ethanol were subjected to a few tests to characterize the pigments in the tapetal region. The reflecting pigment was not dissolved by water, ethanol and xylol; it was dissolved by acetic acid, 10%; oxalic acid, 5%; piperazine hydrate, 2%; iron alum, 5%; picric acid, saturated solution (4 h); sodium hydroxide, 2%; ammonium hydroxide, 10%; hydrochloric acid, 2%. In 5% ammonium hydroxide, the reflecting pigment was not dissolved in 30 min, partially dissolved in 1 h, absent in 2 h. These solubility characteristics are compatible with guanine. Histochemical tests for guanine are unsatisfactory and those tried were negative or ineffective, viz. murexide test, St Hilaire's test, ammoniacal silver nitrate.

The black material in the pigment cells of the tapetal layer was unaffected by all the reagents mentioned in the preceding paragraph, but was slowly bleached by 10% hydrogen peroxide. A melanin is indicated.

DISCUSSION AND CONCLUSIONS

Eyes of dogfish, *Scyliorhinus canicula*, have fixed, i.e. non-occlusible, tapeta. The triangular black field in the lower part of the eye is likewise permanent.

Franz, in his original accounts (1905, 1913), described occlusion of the tapetum in only two species, namely *Acanthia vulgaris* (= *Squalus acanthia*) and *Galeus canis* (= *Galeorhinus galeus*); presumably, these were the only species in which he actually observed the phenomenon. He found that the background of the eye of *Squalus*, in darkness, was highly reflecting; but in sunlight it became uniformly black. Similarly, when he cut open the dark-adapted eye of *Galeorhinus* in a darkened room and exposed it to artificial light or to sunlight, the very bright fundus darkened in an instant, soon reaching maximal darkness. Rochon-Duvigneaud (1943) has remarked that Franz apparently never subjected his ingenious hypothesis of an occlusible tapetum in selachians to experimental proof.

It is possible to illustrate various stages of occlusion of the tapetum in the eye of *Scyliorhinus* by selecting sections at different levels. The result indicates degrees of occlusion; it does not demonstrate that a reversible movement of pigment takes place.

Franz (1931) has figured black ventral fields in the eyes of *Mustelus laevis* (= *M. mustelus*), *Scyllium stellare* (= *Scyliorhinus stellaris*), *Raja clavata* and *R. batis*.

A permanent black ventral field and reflecting fundus may, perhaps, be

features concerned with visual acuity and sensitivity. The ventral field receives maximal illumination from above; the reflecting fundus, from lower levels and from the bottom, where illumination is less. Visual acuity is probably better on a black non-reflecting surface and sensitivity is enhanced by a reflecting tapetum. The dogfish *Scyliorhinus canicula* is nocturnal and lives on the bottom; the organisation of its tapetum appears to be related to its habits. A further study is now being undertaken of tapeta in other elasmobranchs, with a view to relating them to the habits of each species.

SUMMARY

Tapeta were examined of light- and dark-adapted dogfish, *Scyliorhinus canicula*. The tapetum in the eye of this fish is fixed; pigment processes in the tapetum do not move when a fish is changed from dark to light and vice versa. A black ventral field and a bright dorsal fundus are always present.

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STUDIES ON *ECTOCARPUS* IN CULTURE

I. INTRODUCTION AND METHODS OF OBTAINING UNI-ALGAL AND BACTERIA-FREE CULTURES

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In recent years pure cultures of algae have been one of the main sources of material for research on plant metabolism. By far the greatest proportion of this research has been carried out on members of the Chlorophyceae—in fact mainly on members of the genus *Chlorella*. As members of the Chlorophyceae show close resemblances to the higher plants a knowledge of their metabolism is useful in suggesting metabolic pathways in higher plants. However, considerable variations in pigmentation and storage products of the algal classes suggest important differences in metabolism, and it is clearly desirable to have some knowledge of the metabolism of representative members of all the algal classes.

For a reliable investigation of algal metabolism pure cultures should be used whenever possible. Only two members of the Phaeophyceae have hitherto been reported in pure culture (Provasoli, McLaughlin & Droop, 1957) but their growth appears to be too slow to render them of value for physiological or metabolic studies. Species of *Ectocarpus* have been maintained in crude or uni-algal cultures by a number of workers for studies of life history and morphology. When this study was initiated a uni-algal culture of *E. confervoides* (Kütz.) Batt. was already being maintained at University College, London, by Dr J. M. Kain, and as this grew well it was decided to use members of the genus *Ectocarpus* for the present investigation.

Members of this genus are important ship-fouling algae. It is interesting that they show a marked resistance to anti-fouling paints containing copper (Harris, 1943).

The taxonomic position of many species of *Ectocarpus* is in some doubt, but material used for culture work was identified as far as possible from the works of Newton (1931), Hamel (1931–39), Kylin (1947) and Rosenvinge & Lund (1941).

MATERIALS AND METHODS

Although a uni-algal culture of *E. confervoides* was already available it was thought advisable to have another species in uni-algal culture and then if *E. confervoides* proved to be exacting in its requirements a second and perhaps

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less exacting species would be available for purification. For this purpose material of *E. secundus* Kütz. (*Giffordia secunda* (Kütz.) Batt.) was collected from a rock pool at Beer, south-east Devon.

Culture vessels

Cultures were grown in vessels of 'Pyrex' or similar quality glass, the necks of which were plugged with non-absorbent cotton wool. All culture vessels were cleaned by being soaked in chromic acid for 24 h and then being rinsed thirty times with tap water and three times with distilled water to remove traces of chromium.

Media

Natural sea water was used as the basis for most of the culture media and was filtered through paper and stored in the dark for 1 month to 'age' before being used (Harvey, 1941). After storage the pH was 7·7–8·0. The basic culture medium (medium A) was this sea water enriched with potassium nitrate (KNO_3) 2·0 mM (202 mg/l.), potassium phosphate (K_2HPO_4) 0·2 mM (34·8 mg/l.), ferric chloride ($FeCl_3.6H_2O$) 0·01 mM (2·7 mg/l.) and manganese chloride ($MnCl_2.4H_2O$) 0·001 mM (0·2 mg/l.). The phosphate was autoclaved separately from the rest of the medium and added prior to inoculation to prevent precipitation (Kain & Fogg, 1958).

TABLE 1. COMPOSITIONS OF STERILITY-TESTING MEDIA

Medium number	1	2	3	4	5	6
Aged natural sea water (l.)	1	1	1	1	1	1
B.D.H. Bacto-peptone (g)	5·0	5·0	0·5	10·0	1·0	1·0
Ferric phosphate (g)	0·1	0·1	—	—	—	—
B.D.H. Agar (g)	—	20·0	20·0	—	—	20·0
Soluble sodium caseinate (g)	—	—	0·5	—	—	—
Soluble starch (g)	—	—	0·5	—	—	—
Glycerol (ml.)	—	—	1·0	—	—	—
Potassium phosphate (K_2HPO_4) (g)	—	—	0·2	—	—	—
Magnesium sulphate ($MgSO_4.7H_2O$) (g)	—	—	0·05	—	—	—
Glucose (g)	—	—	—	10·0	1·0	1·0
Ferric chloride ($FeCl_3$)	—	—	Trace	—	—	—

All media were adjusted to pH 7·5–8·0 before use. Media 1, 2, and 3 are modified from Spencer (1952), medium 4 from Fogg (1942) and media 5 and 6 adapted from ZoBell (1946).

Occasionally the medium was enriched with organic nutrients. 'Analar' grade chemicals were used where available. Culture materials were normally sterilized by autoclaving at 15 lb. for 15 min. Media containing thermolabile materials were sterilized by Seitz filtering. Six different media, three liquid and three solidified with agar, were used to test for bacterial contamination (Table 1). Not only the culture media but the algal material itself was tested. The test cultures were kept in a dark cupboard at room temperature, usually about 20° C—the optimum for marine

bacteria (ZoBell, 1946), and were examined every few days over a period of 3 weeks. Once pure cultures were obtained little bacterial contamination occurred but fungal contamination did occur on several occasions.

Uni-algal cultures were maintained in medium A to which soil extract was added at 20 ml./l., although it seems likely that soil extract is not necessary for the growth of *Ectocarpus confervoides* or *E. secundus*. Pure cultures of both species were maintained in medium A to which 'Oxoid' dehydrated liver infusion was added at 0·1 g/l.; liver extract was not necessary for growth but did help to indicate the presence of bacterial contamination (Droop, 1954).

Culture apparatus

Three types of culture apparatus were used in all of which the illumination was artificial and continuous.

In culture apparatus 1 cultures were grown around fluorescent lamps at room temperature.

A constant temperature water-bath was used for culture apparatus 2 with illumination from fluorescent tubes in glass sleeves passing through the bath. The cultures were subjected to a constant illumination of 6000–8000 lux and a constant temperature but were not shaken. In order to overcome differences in illumination in different parts of the apparatus the cultures were moved around in the bath at least twice a week.

Apparatus 3, a unit consisting of four thermostatically controlled tanks, has been described fully by Fogg, Smith & Miller (1959). In this, cultures were subjected to controlled temperature and illumination and could be shaken at different speeds.

EXPERIMENTS TO OBTAIN BACTERIA-FREE CULTURES

Various methods for obtaining uni-algal and bacteria-free cultures of marine algae have been described by Schramm (1914), Bold (1942), Chu (1946), Pringsheim (1946), Fish (1950), Provasoli, Pintner & Packer (1951), Spencer (1952), Droop (1954) and Kain & Fogg (1960). The application of these methods to *Ectocarpus* will now be described.

UNI-ALGAL CULTURES

Isolation of single cells

Ectocarpus with its long branching filaments cannot be isolated with micropipettes as is usual in this method. However, filaments of *Ectocarpus* were broken up into short lengths with glass needles and clean lengths isolated. These fragments did not grow, since presumably, as they were only one or two cells long, they did not have active meristematic regions. It was found possible to start cultures from short lengths of filaments with at least one plurilocular sporangium on them, but it was not possible to isolate these sporangia without carrying over epiphytes, especially diatoms, which soon overgrew the *Ectocarpus*. In an attempt to isolate the zoospores, material of *Ectocarpus* bearing plurilocular sporangia and not too densely covered with diatoms was allowed to stand overnight on a moist filter-paper in a sterile Petri dish. The algal material was then transferred to a boiling tube of sterile-

enriched sea water and the spores were soon released giving a suspension of zoospores and diatoms. These zoospores were picked up with micro-pipettes but as soon as they touched the walls of the pipette they lost their mobility and settled down on the inside of the pipette making transfer difficult.

Phototactic isolation

The phototactic isolation method of Droop (1954) is a modification of the previous method. Papenfuss (1935) and Kylin (1943) asserted that the zoospores from the plurilocular sporangia of *Ectocarpus* showed positive phototaxis, but in spite of repeated attempts I found the spores did not show any marked phototaxis.

Dilution

This method did not prove suitable for zoospores of *Ectocarpus* partly because of the ease with which the spores settled on the sides of vessels during the dilution and partly because it was found difficult to obtain a suspension in which spores were more numerous than diatoms.

Plating

As it was unlikely that the zoospores of *Ectocarpus* would germinate on an agar surface the method as normally used (Pringsheim, 1946) was not attempted. Some *Ectocarpus* was left overnight on a moist filter-paper in a Petri dish and the next morning was flooded with sterile culture medium to bring about the release of spores. After 15 min the algal material was removed, a little more culture medium added, and the covered dish placed in the light. Ten days later sporelings of *Ectocarpus* were growing on the filter-paper. Colonies of diatoms were also present but were not spreading very rapidly over the rough filter-paper. The sporelings were examined with a binocular microscope and one which was free from epiphytic diatoms was removed to a tube of sterile medium. In this way a uni-algal culture of *E. secundus* was obtained.

BACTERIA-FREE CULTURES

Although useful preliminary work on algal nutrition may be carried out with uni-algal cultures, for many purposes bacteria-free cultures are essential. Attempts were therefore made to obtain bacteria-free cultures of *Ectocarpus* before any detailed investigation was begun.

The methods for obtaining bacteria-free cultures can be divided into two groups. In the first group the bacteria are eliminated by washing them off the cells or in some way transferring the cells to a bacteria-free medium. The second group of methods aims at killing the bacteria, or at least suppressing their activity whilst the alga continues to grow. Methods of the first type are only successful if there are no bacteria attached to the algal cell walls.

The second group of methods is by far the more effective as these not only remove bacteria in the medium but also allow new cells to grow up without attached bacteria.

Single cell washing

This technique as described by Pringsheim (1946) should be applicable to the zoospores of *Ectocarpus*, but as was previously recorded these proved to be difficult to transfer with micro-pipettes.

Phototactic isolation

The method of Droop (1954) could not be applied because under the conditions employed in this investigation the zoospores were not sufficiently phototactic.

Dilution and plating

For the dilution method to be successful it was necessary that zoospores should be more abundant than bacteria in the suspension to be diluted, a situation that was never obtained. As the zoospores did not survive very long on the moist filter-paper in a Petri dish it seemed unlikely that they would grow on an agar surface.

Pringsheim (1946) has commented that contaminating bacteria are always attached to the walls of members of the Phaeophyceae. Tests made on material of *Ectocarpus* showed a number of bacterial types in the medium and additional types on the alga itself.

Ultra-violet irradiation

Prolonged exposure to ultra-violet irradiation is lethal to most living organisms, but the lethal dose varies from one organism to another and is difficult to predict for any specific organism. It has been shown that doses of ultra-violet irradiation below the lethal level may have very marked effects on living organisms (Redford & Myers, 1951). However, ultra-violet irradiation has been successfully used to obtain pure cultures of algae and does not appear to have had any deleterious effects. This technique has been successful with a filamentous blue-green alga (Fogg, 1951) and was therefore tried with *Ectocarpus*.

A young uni-algal culture of *E. confervoides*, consisting mainly of sporelings 5–7 mm long, was transferred to a quartz boiling tube which was fixed 15 cm from a 'Hanovia' 12 in. jacketed ultra-violet lamp (wavelength 2537 Å). The lamp was switched on and the quartz tube well shaken every $\frac{1}{2}$ min. After 5 min of irradiation samples were taken aseptically from the quartz tube and culture tubes of each of eight different nutrient sea water media were inoculated. Further samples were taken after 10, 20 and 30 min irradiation. After

8 days illumination all media containing organic nutrients showed bacterial growth. Thirty minutes irradiation had not killed all the bacterial contaminants and tests showed at least three bacterial types were still present. Although this experiment had not produced a pure culture even the longest period of irradiation appeared to have had no ill effects on *Ectocarpus*, and it was thought worth while subjecting this material to even longer periods of irradiation. The times of irradiation in the next experiment were 25, 35 and 45 min and the material was a culture of sporelings grown up from material subjected to 30 min irradiation in the previous experiment. After 45 min irradiation the tips of the filaments of the sporelings showed a very marked bleaching. All cultures grew but the material subjected to 45 min irradiation gave positive contamination tests and the contaminants appeared to be the same bacterial types as were present at the beginning of the experiment. *E. secundus* was also subjected to ultra-violet irradiations. The experimental procedure was the same as in the previous experiments. Total irradiation times were 20, 30 and 40 min. All material subjected to 40 min irradiation died and all media containing organic nutrients showed bacterial growth, even those in which the alga had died. Forty minutes irradiation had killed the alga but not all the contaminants, although the number of contaminating types was again reduced.

Antibiotics

As with ultra-violet irradiations there have been some reports that a sub-lethal dose of antibiotics can have mutagenic effects. Nevertheless, several workers (Fish, 1950; Provasoli *et al.*, 1951; Spencer, 1952; and Provasoli *et al.*, 1957) have used antibiotics singly or in mixtures to obtain bacteria-free cultures of marine algae.

A sterile solution was prepared from 'Glaxo' crystalline sodium penicillin G and was added to sterile culture medium A to give a final penicillin concentration of 4000 units/ml. Material of *E. confervoides* subjected to 30 min ultra-violet irradiation was subcultured and the young sporelings from this culture transferred to the penicillin solution. Subcultures were made from the penicillin after 9, 21 and 45 days and subcultures made into media containing organic nutrients did not show any bacterial growth. Before these cultures could be thoroughly tested for contamination it was important that all traces of penicillin should be removed. Further subcultures were made every 7–10 days and at each subculture only a small number of young sporelings was used as inoculum. After five such serial subcultures the cultures were tested for bacterial contamination and both culture medium and algal material gave negative results. *E. secundus* was treated in a similar way. In this experiment a range of concentrations of penicillin was used. Once again a culture from an ultra-violet irradiation experiment was used to provide sporelings for treatment with penicillin. The concentrations of penicillin and lengths

of treatment are given in Table 2. Bacteria-free cultures of *E. secundus* were successfully obtained by this method.

The strain of *E. confervoides* arising from material subjected to 4000 units/ml. of penicillin for 9 days has been used for investigations to be described in subsequent papers.

TABLE 2. TREATMENT OF *ECTOCARPUS SECUNDUS* WITH PENICILLIN

c, contaminated; F, bacteria-free

No. of days in penicillin	Penicillin concentration (units/ml.)		
	1000	5000	10,000
1	c	c	c
2	c	c	c
4	c	c	F
8	c	F	F
15	F	F	F

I am deeply indebted to Prof. G. E. Fogg for his stimulating advice and continual encouragement during this investigation. I would also like to express my thanks to Dr J. M. Kain for providing me with the uni-algal culture of *E. confervoides*. This work was carried out during the tenure of a Fishery Research Training Grant for which I am indebted to the Development Commission.

SUMMARY

Details are given of the culture apparatus and methods found successful for the laboratory culture of species of *Ectocarpus*. In attempts to obtain uni-algal cultures of this genus several common methods were tried but only one of them, a modification of the plating technique, proved successful. Methods of washing and dilution were unsuccessful in giving bacteria-free cultures, presumably due to bacteria closely adhering to the cell walls. Ultra-violet irradiation was also unsuccessful but treatment with penicillin gave bacteria-free cultures of *E. confervoides* and *E. secundus* (*Giffordia secunda*).

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STUDIES ON *ECTOCARPUS* IN CULTURE

II. GROWTH AND NUTRITION OF A BACTERIA-FREE CULTURE

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(Text-figs. 1–7)

Part I of this series (Boalch, 1961) described how bacteria-free cultures of two species of *Ectocarpus* were obtained. Here experiments on the growth and nutrition of a bacteria-free culture of *E. confervoides* (Kütz.) Batt. are described.

The usual methods of estimating growth in laboratory cultures of algae are: (i) by counting the number of cells per unit volume, (ii) from optical density—of the culture or a pigment extract, (iii) from total dry weight, (iv) from total cell volume calculated from cell measurements or packed cell volume.

Cell counting is most suitable for unicellular algae, but it has been used for early stages of filamentous algae (Boney, 1960), and also for a blue-green alga after the filaments have been broken into short lengths (Fogg, 1944). Measurement of optical density is again most suitable for unicellular algae. The optical density of extracted pigments has been used, but this assumes that the pigment content per cell remains constant throughout the growth cycle. Increases in dry weight may be due to thickening of cell walls or accumulation of storage products and may occur without any increase in cell numbers. The total cell volume method is again best applied to unicellular algae, when volume can be calculated from cell measurements and cell numbers. Whenever possible more than one method of estimating growth should be used. Pearsall & Loose (1937) in their study of *Chlorella* estimated growth by cell counts, dry weight and cell volume. Cell counts and optical density have been used by Spencer (1954), Droop (1957*a*), and Kain & Fogg (1958*a*, *b*) to study the growth of marine algae. A full discussion of the growth of a unicellular alga in pure culture has been given by Myers (1953).

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METHODS

Estimation of growth

Estimation of the growth of *Ectocarpus* by cell-counting was not possible, and as the filaments were not easily fragmented the filament length method of Fogg (1944) could not be used. Determination of the optical density of the culture was also impracticable. Measurement of extracted pigments was attempted, but it was found that extraction was incomplete and the percentage of the total pigment that was extracted depended upon the age of the culture. At first dry weights were not reliable. If the algal material was not washed large amounts of salt were left in the material and rendered it hygroscopic. When the algal material was washed with distilled water care had to be taken to prevent too much leaching. The method finally adopted and found quite reliable was as follows. Using a vacuum pump algal material from one culture was filtered off into a sintered glass crucible of porosity 40–90 μ. Still using the vacuum pump the material in the crucible was rapidly washed with distilled water and the crucible quickly transferred to the oven at 100° C. After 24 h in the oven the crucibles were allowed to cool in a desiccator for 1 h and then weighed as rapidly as possible.

As a culture was used up every time a sample was taken, the number of cultures needed to study the growth of *Ectocarpus* was large. It was found impracticable to take more than two replicate samples every time a dry-weight determination was required. These duplicates sometimes showed considerable divergences, especially in the early stages of growth when the amounts of algal material being weighed were small. With only two samples it is difficult to apply any statistical analysis to the results and therefore no such application has been attempted. The usual method adopted when studying the effect of a series of conditions on the growth of *Ectocarpus* was to take five samples, each in duplicate, during the growth cycle under each of the conditions investigated. The period of growth usually extended over 6–8 weeks.

Inoculation of cultures

The problem of introducing equal amounts of a filamentous alga into each of a large number of culture vessels is a difficult one to overcome. When this investigation was begun it was thought that it would be possible to use a suspension of zoospores as inoculum and carry out inoculations in the way normally used for unicellular algae. However, the transfer of zoospores of *Ectocarpus* proved difficult (Boalch, 1961) and a method had to be devised in which uniform inoculation could be made with the filamentous algal material itself. When large numbers of tubes were to be inoculated stock material was grown up in 1 l. of medium in a 'penicillin' flask. Material was then transferred aseptically to a boiling tube of sterile sea water and cut up into uniform pieces with a sterile Borradaile needle and these pieces used to inoculate the experimental tubes. In addition to the experimental tubes a number of test-tubes of sea water were inoculated in a similar way and the contents of these tubes then filtered off for estimation of the initial dry weight. An inoculating chamber, previously sterilized with an ultraviolet lamp, was used for all subculturing.

Media

All culture media were enriched with potassium nitrate and potassium phosphate at the levels given for medium A in the previous paper in this series (Boalch, 1961). Iron and manganese, unless added in trace element mixtures, were also added at the levels used for medium A. Full details of the chemical composition of all media are given in Table 1.

TABLE 1. COMPOSITION OF CULTURE MEDIA

Unless otherwise stated all units in the table are weights in mg. Medium D_1 is medium S20 of Droop 1955 and medium F is the 12S50 of Droop, 1958 but with some of the organic additions omitted.

Medium no.	A	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	C_1	C_2	D_1	E_1	E_2	E_3	F_1	F_2	G_1
Aged natural sea water (l.)	1	1	1	1	1	1	1	1	1	1	—	—	—	—	—	—	—	—	—
Glass-distilled water (l.)	—	—	—	—	—	—	—	—	—	—	1	1	1	1	1	1	1	1	1
KNO_3	202	202	202	202	202	202	202	202	202	202	202	202	202	202	202	202	202	202	202
K_2HPO_4	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8	34·8
$FeCl.6H_2O$	2·7	2·7	2·7	2·7	2·7	2·7	2·7	—	—	—	—	—	—	—	—	—	—	—	—
$MnCl.4H_2O$	0·2	0·2	0·2	0·2	0·2	0·2	0·2	—	0·2	—	—	0·2	—	0·2	—	—	—	—	—
NaCl	—	—	—	—	—	—	—	—	—	—	23,477	23,477	15,000	15,000	30,000	15,000	15,000	30,000	24,500
$MgCl_2.6H_2O$	—	—	—	—	—	—	—	—	—	—	10,634	10,634	2,500	2,500	5,000	2,500	2,500	5,000	9,800
$Na_2SO_4.10H_2O$	—	—	—	—	—	—	—	—	—	—	8,884	—	—	—	—	—	—	—	7,300
$CaCl_2$	—	—	—	—	—	—	—	—	—	—	1,102	—	—	—	—	—	—	—	400
$CaSO_4.2H_2O$	—	—	—	—	—	—	—	—	—	—	—	—	500	500	1,000	1,000	500	1,000	—
K_2SO_4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	850
Na_2 E.D.T.A.	—	—	—	—	—	—	—	—	—	20	20	—	20	—	20	40	50	50	20
$NaHCO_3$	—	—	—	—	—	—	—	—	—	—	192	—	—	—	—	—	—	—	—
KCl	—	—	—	—	—	—	—	—	—	—	664	—	375	375	750	750	375	750	—
KBr	—	—	—	—	—	—	—	—	—	—	96	—	15	15	15	30	32·5	32·5	32·5
KI	—	—	—	—	—	—	—	—	0·06	—	—	0·06	—	0·06	—	—	0·025	0·025	0·025
NaF	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—
H_3BO_3	—	—	—	—	—	—	—	—	—	26	—	—	—	—	—	—	—	—	—
$FeSO_4.7H_2O$	—	—	—	—	—	—	—	—	—	0·7	1·28	—	0·7	—	0·7	1·4	2·5	2·5	0·7
$FeC_6H_5O_7$	—	—	—	—	—	—	—	—	0·54	—	—	0·54	—	0·54	—	—	—	—	—
$AlCl_3.6H_2O$	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·15	0·15	0·15
$Al(SO_4)_3$	—	—	—	—	—	—	—	—	3·0	—	0·3	3·0	0·25	3·0	0·25	5·0	—	—	—
As_2O_3	—	—	—	—	—	—	—	—	0·03	—	—	0·03	—	0·03	—	—	—	—	-
$BaCl_2.2H_2O$	—	—	—	—	—	—	—	—	0·09	—	—	0·09	—	0·09	—	—	—	—	—
$CoSO_4.9H_2O$	—	—	—	—	—	—	—	—	—	0·03	0·03	—	0·03	—	0·03	0·06	0·002	0·002	0·03
$CuSO_4.5H_2O$	—	—	—	—	—	—	—	—	0·04	0·005	0·014	0·04	0·005	0·04	0·005	0·01	0·019	0·019	0·005
$LiCl.H_2O$	—	—	—	—	—	—	—	—	1·0	—	0·1	1·0	0·05	1·0	0·05	0·1	0·05	0·05	0·05
$MnSO_4.H_2O$	—	—	—	—	—	—	—	—	—	2·0	2·24	—	2·0	—	2·0	4·0	0·155	0·155	2·0
$NaMoO_4.2H_2O$	—	—	—	—	—	—	—	—	—	0·5	0·6	—	0·5	—	0·5	1·0	0·001	0·001	0·5
$NaSiO_3.9H_2O$	—	—	—	—	—	—	—	—	10	—	—	10	—	10	—	20	—	—	—
RbCl	—	—	—	—	—	—	—	—	0·28	—	0·1	0·28	0·05	0·30	0·05	0·1	0·1	0·1	0·1
$SrCl_2.6H_2O$	—	—	—	—	—	—	—	—	—	—	40	—	5·0	5·0	5·0	10	6·5	6·5	6·5
$ZnSO_4.7H_2O$	—	—	—	—	—	—	—	—	0·01	10	8·9	0·01	10	0·01	10	20	0·022	0·022	10
Soil extract	—	20 ml	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
'Oxoid' liver extract	—	—	100	—	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—
'Oxoid' yeast extract	—	—	—	—	—	—	100	—	—	—	—	—	—	—	—	—	—	—	—
'Difco' Bacto peptone	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	-	—	—	—
'Difco' Bacto tryptone	—	—	—	—	—	100	—	—	—	—	—	—	—	—	—	—	-	—	—
Vitamin B_{12} (μg)	—	—	—	0·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Agar (g)	—	—	—	—	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—

EXPERIMENTS

GROWTH AND VIABILITY OF CULTURES

Growth curves from two experiments in which cultures were grown in medium A in unshaken boiling tubes are illustrated in Fig. 1 A, B. Duplicate samples were taken for each reading and the limits of the duplicates are indicated on the dry weight graph, but only the means are plotted on the log. graph. The lag phase is of 5–7 days duration and although during this period there is no increase in dry weight, there is considerable activity, for during this period numerous plurilocular sporangia are formed. The zoospores

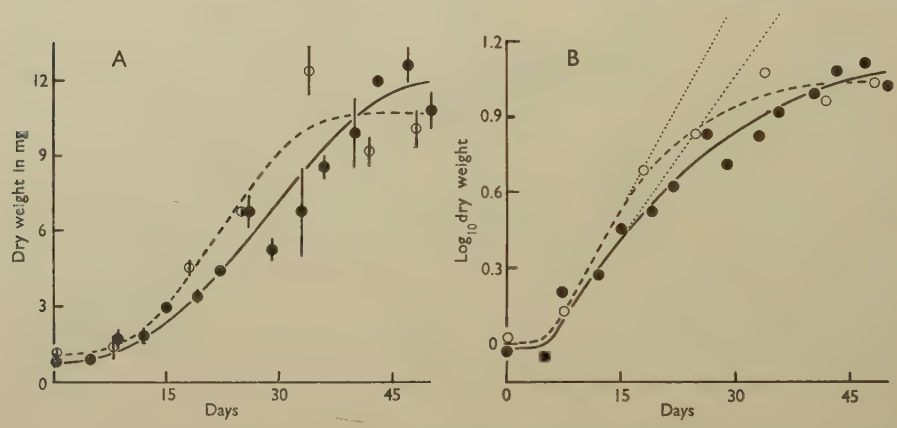

Fig. 1. Growth curves (medium A). (A) Growth of bacteria-free cultures in two experiments (○—○ and ●—●). (B) Logarithmic growth curves for the same two experiments (dotted lines are extrapolations of the early part of logarithmic growth from which k was calculated.

formed in these sporangia either are released into the medium and settle on the walls of the culture vessel and germinate, or more usually they germinate *in situ*. In some experiments a slight decrease in dry weight has been noted towards the end of the lag phase. This is probably due to the zoospores having recently been released and thus incompletely sampled. The second phase of growth is not truly exponential as with most micro-organisms. Instead of the slope of the graph being constant over a considerable period it falls off gradually. Tentative lines have been dotted in for the slope of the early part of the logarithmic curves to allow the relative growth constant k to be calculated. During the logarithmic phase growth is expressed by the following equation

$$n_t = n_0 e^{kt}.$$

When n_t is the cell number (or some other measure of the algal material in the culture) at the time 0 and n_t is the cell number at time t, k is the relative

growth constant and e the base of natural logarithms. This equation may also be expressed as

$$\log n_t = \log n_0 + kt.$$

The relative growth constant k is usually expressed as $\log_{10}$ units/day. The values of k for the two experiments are 0·04 and 0·05 giving an average value for k of 0·045. This is equivalent to a doubling time of 6·5 days, i.e. during the early stages of rapid growth *E. confervoides* doubles its dry weight every 6–7 days.

Viability of cultures

Cultures over 1 year old proved to be still viable when subcultured. *Ectocarpus* inoculated into medium A and kept in the dark did not grow but was still viable after 150 days. Cultures on medium A solidified with agar showed considerable growth in the light and were still viable after 100 days, but soon after this the cultures dried up and were dead by 130 days. Similar cultures in the dark did not grow but remained viable for at least 100 days.

INORGANIC NUTRITION

Nitrate requirements

A number of experiments was carried out using medium A in which the amount of added nitrate was varied. Levels ranging from 0·1 to 2·0 mM (10–202 mg KNO_3/l.) were used and a typical example of the results is given in Fig. 2A. Both the rate of growth and the final yield increased as nitrate was increased up to a level of 0·5 mM. However, when the nitrate was increased to 1·0 mM, or more, the rate of growth was rather less for the first 35 days but after about 50 days the final yield was the same. A further experiment was carried out in which the potassium nitrate was replaced by similar levels of sodium nitrate. The results were similar to those for potassium nitrate and it therefore seems unlikely that the reduced growth rate was due to an excess of potassium ions.

Phosphate requirements

Similar experiments were carried out with concentrations of potassium phosphate ranging from 0·025 to 0·2 mM (4·4–34·8 mg K_2HPO_4/l.) (Fig. 2B). The growth rate and final yield were increased by additions of up to 0·1 mM. phosphate but increases above this show a fall in growth rate for the first 35 days and after 50 days the final yields were less. The pattern was thus not quite the same as that for nitrate. Although the phosphate was autoclaved separately and added to the culture medium just prior to inoculation there was some precipitation at levels of 0·1 mM and above and this may have been responsible for the inhibition at these levels. This precipitate disappeared during growth but it was possible that some other essential elements were

brought out of solution by this precipitation and did not redissolve with the phosphate.

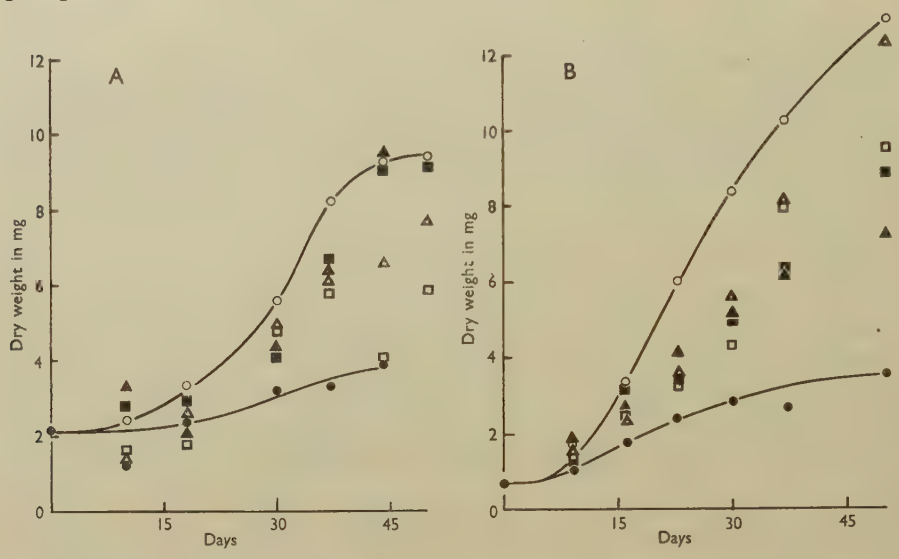

Fig. 2. Nitrate and phosphate. (A) Effects on growth of additions of potassium nitrate (KNO_3): ●, no nitrate; □, 0·1 mM nitrate; △, 0·25 mM; ○, 0·5 mM; ▲, 1·0 mM; ■, 2·0 mM. (B) Effect on growth of additions of potassium phosphate (K_2HPO_4): ●, no phosphate; □, 0·25 mM phosphate; △, 0·05 mM; ○, 0·1 mM; ▲, 0·15 mM; ■, 0·2 mM.

Iron requirements

The additions of iron, added as a freshly made solution of ferric chloride at concentrations of from 0·01 to 0·04 mM (2·7–10·8 mg $FeCl_3.6H_2O$/l.) had no effect upon the rate of growth or the final yield. The growth with these additions of iron was the same as in the control to which no iron was added. Of course at the pH of these experiments most of the inorganic iron would be in an insoluble form.

Manganese requirements

Manganese was added as a freshly prepared solution of manganese chloride at levels of 0·001–0·004 mM (0·2–0·8 mg $MnCl_2.4H_2O$/l.) and produced increased growth and final yield at all levels within this range (Fig. 3A).

ORGANIC NUTRITION

Additions of complex organic mixtures such as yeast extract and liver extract at 0·1 g/l. and soil extract at 20 ml./l. to the basic medium A gave increases in final dry weight of from 10 to 25% but peptone at 0·1 g/l. considerably reduced the final yield. Growth in medium A enriched with vita-

min B_{12} (0·1 μg/l.) or soil extract (20 ml./l.) was slightly better than in the unenriched medium (Fig. 3 B). It has been shown that some algae will grow in the dark and utilize organic compounds as carbon sources. The following organic carbon sources were added to medium A at levels of 1·0 g/l. prior to autoclaving: sodium glycerophosphate, hexose 1·6 diphosphate (calcium salt), maltose, mannitol, D-mannose, lactose, dextrose, D-leavulose and sodium acetate. After inoculation some cultures of each media were placed

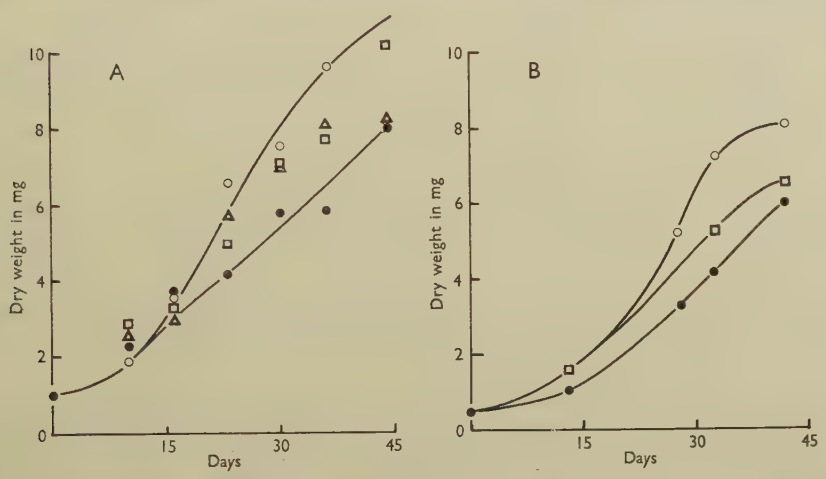

Fig. 3. Manganese and organic additions. (A) Effect on growth of additions of manganese chloride (MnCl): ●, no manganese; □, 0·001 mM manganese; △, 0·002 mM; ○, 0·004 mM. (B) Growth with additions of organic compounds: ●, normal medium A; □, medium A with vitamin B_{12} at 0·1 μg/l.; ○, medium A with soil extract at 20 ml./l.

in a dark cupboard and others were kept in continuous illumination. All cultures were at room temperature. The cultures in the light grew well but none of those in the dark showed any growth. These organic compounds clearly had no inhibitory effect in the light but they could not be utilized for chemotrophic growth.

PHYSICAL CONDITIONS

Salinity

In order to investigate the growth of *Ectocarpus* in media of different salinities some natural sea water, of approximate salinity 35 ‰, was concentrated to half its original volume by vacuum distillation. By diluting with distilled water this concentrate was used to make up salinities of 2, $1\frac{1}{2}$, $1\frac{1}{4}$, 1, $\frac{3}{4}$, $\frac{1}{2}$, and $\frac{1}{4}$ that of natural sea water. These, together with some distilled water, to give a salinity of zero, were enriched at the levels normally used for medium A. Cultures were grown in these media at 20 °C and samples taken after 50 and 67 days (Fig. 4 A). There was no growth at salinities of 2,

$\frac{1}{4}$, and 0 times that of natural sea water. In a further experiment salinities of $1\frac{3}{4}$, $1\frac{1}{2}$, $1\frac{1}{3}$, 1, $\frac{3}{4}$, $\frac{1}{2}$ and $\frac{1}{4}$ those of natural sea water were used. Larger culture vessels were used for this experiment and the cultures were shaken in apparatus 3 (Boalch, 1961) at 15° C for 87 days (Fig. 4B). Although the results of the two experiments cannot strictly be compared it should be noted that at 20° C the maximum dry weight was obtained at a salinity higher than that of natural sea water, whereas at 15° C the maximum yield was at a salinity close to that of natural sea water.

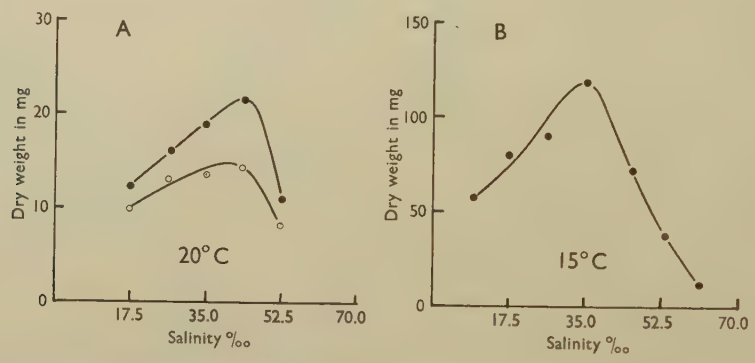

Fig. 4. Yields in media of different salinity. (A) At 20° C: ○, after 50 days; ●, after 67 days. (B) After 87 days at 15° C.

Optimum pH

In these experiments the buffer 'tris', (hydroxymethyl)aminomethane, was added to medium A at 500 mg/l. Cultures were grown at 20 °C at initial pH's of 9·0, 8·5, 8·0, 7·5, and 7·0. The results are illustrated graphically in Fig. 5 where both the initial and final pH are given. The optimum initial pH was 8·0 and during the course of the experiment the pH of these cultures rose to 8·8.

Light intensity

To study the effect of a range of light intensities on the growth of *Ectocarpus*, cultures were grown in 100 ml. conical flasks in culture apparatus 3 at 20° C. The light intensity was varied by fixing layers of black nylon net under the culture vessels, which were shaken at 88 oscillations per minute (Fogg, Smith & Miller, 1959). The investigation was carried out in two stages with a different maximum light intensity in each experiment. Unfortunately, due to differences in the condition of the inoculum, the two parts of the experiment (Fig. 6C, D) cannot be compared directly. However, in each experiment the relative growth constants at the three light intensities were similar and there were only small differences in the final yield, but there were marked differences

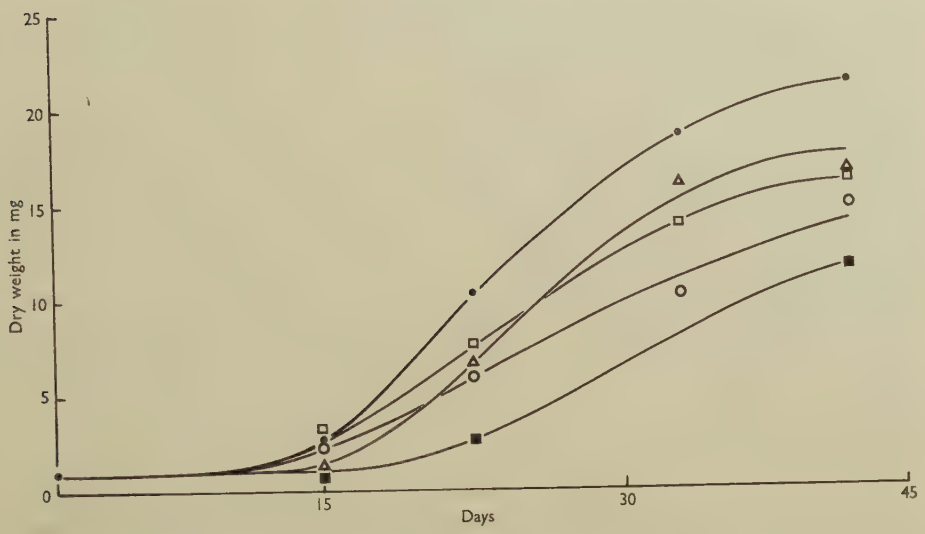

Fig. 5. Growth in media of different pH. Both initial and final pH values are given: ■, 7·0–8·0; □, 7·5–8·1; ●, 8·0–8·8; ○, 8·5–9·1; △, 9·0–9·4.

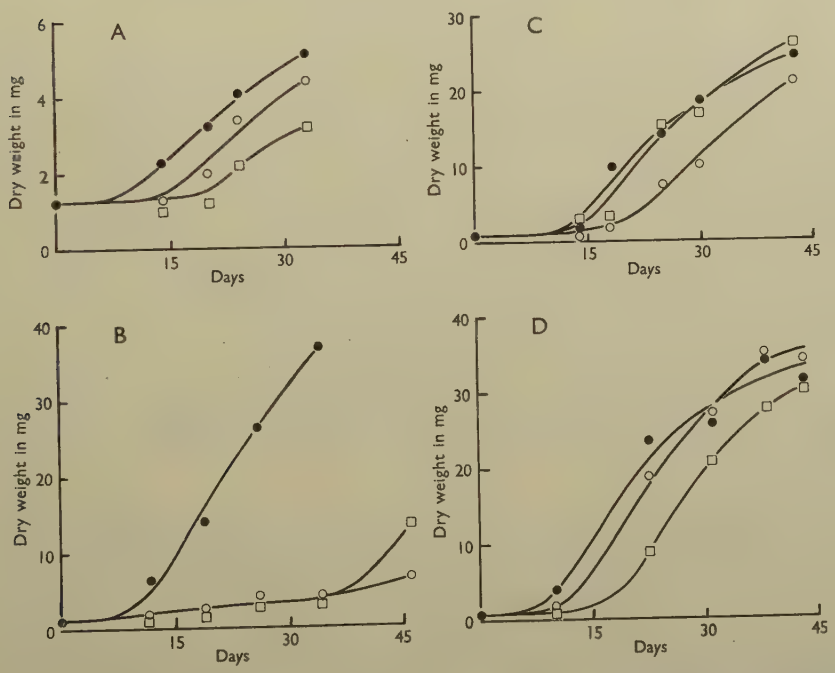

Fig. 6. Light and temperature; (A) Growth at: ●, 20° C; ○, 25° C; □, 10° C in boiling tube cultures. (B) Growth at: ●, 20° C; ○, 25° C; □, 10° C in shaken conical flasks. (C) growth at: □, 1500 ft. candles; ●, 600 ft. candles; ○, 250 ft. candles in shaken conical flasks. (D) Growth at: ●, 700 ft. candles; ○, 250 ft. candles; □, 125 ft. candles in shaken conical flasks.

in the length of the lag phase. In both experiments the lag was shortest at 600–700 ft. candles (6500–7500 lux.).

Optimum temperature

Three experiments were carried out on the effect of temperature on growth, the first two of these are illustrated graphically in Fig. 6. All experiments were carried out in culture apparatus 3. In the first experiment the cultures were grown in unshaken boiling tubes and the temperatures were 10, 20, 25 and 30° C. All the cultures at 30° C died, but at the other three temperatures the relative growth constants were similar although there were variations in the length of the lag phase (Fig. 6A). Shaken 100 ml. conical flasks were used as culture vessels in the second experiment and the temperatures were 10, 20 and 25° C. In this case there was very little growth at 10° C and hardly any growth at 25° C (Fig. 6B). Temperatures of 15 and 20° C were used in a third experiment and no difference was found in lag phase, relative growth constant and final yield at these two temperatures. The results indicate that *Ectocarpus* will grow at temperatures between 10 and 25° C but will not grow at 30° C. The optimum temperature for the growth of *Ectocarpus* under bacteria-free conditions is between 15 and 20° C.

ARTIFICIAL SEA WATER

Although aged natural sea-water forms a good basic medium for many marine algae it has the disadvantage that the concentrations of the nutrients already present are variable. Further, whilst natural sea water is being used as the basis for culture media, it is impossible to ascertain whether or not the alga has a requirement for a particular organic nutrient as so many organic compounds are present in sea water. The experimental investigation of artificial sea waters was aimed at finding a chemically defined artificial medium in which *Ectocarpus* would grow as well as in natural sea-water media. As the literature on sea-water media has been reviewed by Provasoli, McLaughlin & Droop (1957) discussion of it is unnecessary here.

As *Ectocarpus* grew well in natural sea water, without the addition of organic compounds, it was thought best that at first attempts should be made to culture it in an artificial medium without any added organic compounds.

In the first experiment the medium was that of Chu (as given in Harvey, 1955), this is C_2 in Table 1. The medium C_1 was similar but was made up with a different trace element mixture. These media were also mixed with equal volumes of natural sea water to give two further media. Growth in these four media was compared with that in medium A. In C_1 there was no visible growth and only very little growth in C_2 but there was good growth in all media containing natural sea water. The next experiment used media based on those of Droop (1955). In addition medium A was enriched with two

different trace element mixtures (A_8, A_9) to test if these mixtures were in any way inhibitory. The six media used were A, A_8, A_9, C_2, D_1 and E_1. Again there was good growth in media containing natural sea water and little growth in medium C_2. There was some growth in media D_1 and E_1 but not as good as that in natural sea water and on subculturing into the same medium again the culture died. These media of Droop have a salinity of about 16 ‰ and it has been shown previously in this paper that the optimum salinity for *Ectocarpus* at 20° C is above that of the natural sea water, i.e. above 35 ‰. In the next experiment the salinity of some media was doubled (E_2, E_3) and growth compared with that in media A and D_1. In all the artificial media there was a long lag phase, no growth being visible until after 30 days.

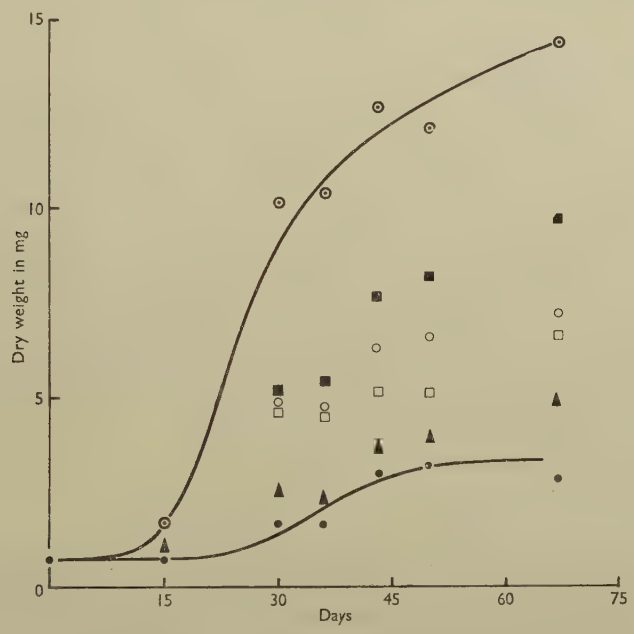

Fig. 7. Artificial media. ⊙, Natural sea-water medium A, ●, D_1; ■, E_2; ▲, F_1; □, F_2; ○, F_3. For details of media see Table 1.

However, there was growth in the two media with the higher salinity but the final yield was only about half of that in medium A. These media were then tried with additions of organic compounds. Media D_1 and E_2 were made up and each was divided into three portions. One portion was used direct, one was enriched with liver extract at 0·1 g/l. and the third portion was enriched with vitamin B_{12} at 0·1 μg/l. and the final yields in the six media compared. Once again the higher salinity media showed much better growth than those at the lower salinity. At the low salinity the addition of the nutrients reduced

the yield, but at the higher salinity there was no marked difference between the three media. The addition of further trace elements to medium E_2 enriched with liver extract had no effect on growth. A further medium of Droop (1958) was then investigated. This was prepared as described by Droop, (F_1), and also with the salinity doubled (F_2, F_3). Growth in these media was compared with growth in A, D_1 and E_2, the results being illustrated in Fig. 7. Once again growth in the low-salinity media, D_1 and F_1, was poor but even when the salinities were doubled the yields were at the maximum only two-thirds of that in the natural sea-water medium. In one further experiment vitamins were added to F_2, in one case vitamin B_{12} was added at 0·1 μg./l. and a complex vitamin mixture was added to some more medium at two different levels. This mixture consisted of 200 mg chlorine, 200 mg nicotinamide, 200 mg calcium pantothenate, 0·4 mg folic acid, 400 mg inositol, 50 mg para-amino-benzoic acid, 50 mg pyridosine, 50 mg riboflavin and 100 mg thiamin all dissolved in 100 ml. of distilled water, and was added at levels of 0·4 and 1·0 ml. of stock solution per litre of culture medium. The vitamin mixture did not have any marked effect on growth and although the addition of vitamin B_{12} did increase the yield the final yield was still only just over half of that in natural sea water.

These experiments with artificial sea waters have shown that *Ectocarpus* will show some growth in quite a range of artificial media and it grew quite well in Droop's media when the salinity was doubled and brought nearer to that of natural sea water. The additions of vitamin B_{12} and liver extract brought about some improvement in the growth of *Ectocarpus* in these artificial media but the yields were never more than two-thirds of that obtained in natural sea water media.

DISCUSSION

It is of interest to compare the results of this investigation with the findings of other workers in similar fields. It has already been pointed out that the growth curve was rather as expected but was not as well defined as for most micro-organisms. This is probably in part due to the nature of the alga itself. The growth of *Ectocarpus* is not simply a matter of each cell dividing into two. In the erect system of *Ectocarpus* cell division is limited to certain meristematic zones which are usually near the base of branches. If the number of meristematic zones remained constant, growth would be linear and not logarithmic. However, the number of meristematic zones increases as new branches are formed and new sporelings develop. The growth of an *Ectocarpus* culture is thus intermediate between linear and logarithmic growth. Further as the meristematic zones become hidden at the base of the tangled filaments the amount of light and nutrients they receive is reduced and the rate of cell division is probably reduced. In shaken cultures the algal material

tends to roll into a ball so that the effect of shaking is not as advantageous as might be expected.

The best growth of *Ectocarpus* was brought about by the addition of 0·5 mM potassium nitrate to natural sea water. In 20 ml. of culture medium this gave an increase, over the control, of 5 mg dry weight. This is an increase of 250 mg dry weight/l. for the addition of 7 mg of nitrogen (0·5 mM). Assuming complete utilization of the nitrogen, this would correspond to a nitrogen content of 2·8 % on a total dry-weight basis. An estimation of the nitrogen content of a culture of *Ectocarpus* grown in a medium enriched with 2·0 mM nitrate (Fogg & Boalch, 1958) gave 7 mg of cellular nitrogen in a culture of 1 l. The dry weight of this culture was approximately 300 mg and the nitrogen content of the algal material was thus 2·3 % of the dry weight. When 0·25 mM nitrate was added the calculated nitrogen content of the algal material would be 2·3 % of the dry weight. At 0·1 mM the nitrogen content could not have been greater than 1·5 % and it must be assumed that at this level the culture was growing under conditions of acute nitrogen deficiency. Black (1948) gives the total nitrogen content of *Laminaria* spp. as 2·5–0·7 % of the dry weight depending on the time of year and conditions of growth. Milner (1953) found the nitrogen content of *Macrocystis* as 6·6 % of the ash-free dry weight, or 4·2 % of the total dry weight. The minimum nitrate needed for maximum crop of *Asterionella japonica* and *Isochrysis galbana* (Kain & Fogg, 1959*a*, *b*) was of the order of 1·0 mM but neither of these planktonic algae was bacteria-free.

Maximum growth of *Ectocarpus* was obtained with 0·1 mM phosphate and although the cultures had not reached the stationary phase after 50 days by this time addition of phosphate had increased the dry weight by 500 mg/l. The yields obtained with the various levels of phosphate give phosphorus figures of 0·62 % of the dry weight at 0·1 mM phosphate, 0·45 % at 0·05 and 0·32 % at 0·025 mM. These figures all assume complete utilization of the available phosphorus, and as already noted there was some precipitation at the highest level of phosphate, but as no estimates of the phosphorus content of the algal material were made these values cannot be checked. Kain & Fogg (1958*b*) found the minimum need of phosphate for *Isochrysis* was of the order of 0·03 mM.

These experiments show that the requirements for the best growth of *Ectocarpus* were 0·5 mM nitrogen and 0·1 mM phosphorus giving a ratio for N/P of 5/1 and not the 20/1 often quoted for marine algae. Cooper (1937) found a ratio of 16·3/1 in marine phytoplankton and 20/1 in the sea, while Redfield (1934) gave a ratio of 18/1 in plankton.

It was surprising that the addition of ferric chloride to natural sea water had no effect on growth. Harvey (1937) showed that the iron requirements of a marine diatom were more than was directly available to it in a soluble form in the sea water. He also showed that the diatom could take up iron that was

only present in a precipitated form and that the removal of this precipitate limited growth. However, the precipitate could be replaced by ferric citrate. Goldberg (1952) studied the iron requirements of *Asterionella japonica* and found no matter how he added the iron to sea water there was a considerable amount of hydrolysis. It appeared that *Asterionella* needed particulate iron and could not utilize iron that was only present in the soluble form. As the addition of freshly prepared ferric chloride solution to sea water had no effect on the growth of *Ectocarpus* it must be assumed that, either the sea water contained sufficient iron for the growth of this alga under the experimental conditions of the investigation, or that the alga was unable to utilize iron that was present in an insoluble form.

The addition of manganese to sea water stimulated the growth of *Ectocarpus* up to the highest concentration used, 0·004 mM manganese. Harvey (1947) found additions as low as 0·5–2·0 mg of manganese per cubic metre (0·00001–0·00004 mM) gave increases in the growth of marine algae.

The range of salinities that *Ectocarpus* would tolerate was quite wide, being from $\frac{1}{2}$ to $1\frac{1}{2}$ times the salinity of natural sea water at 20 °C and $\frac{1}{4}$ to $1\frac{3}{4}$ times the salinity of sea water at 15° C. The results of the two experiments on salinity were not completely comparable as the experiment at 20° C was carried out with unshaken boiling tubes, whereas shaken conical flasks were used for the experiment at 15° C. It is, however, interesting that the optimum salinity differed at the two temperatures. At 20° C the greatest yield would have been given by a medium with a salinity slightly higher than that of natural sea water, but at 15° C the highest yield would have been given at a salinity less than that of natural sea water. Ritchie (1957) found the optimum salinity for the growth of two marine fungi decreased with decreasing temperature and experiments indicate that this is also the case with *Ectocarpus*. Biebl (1952) asserted that intertidal algae would tolerate salinities of from 10 to 300 % that of natural sea water, and that species that occur at or near low water will tolerate 40–200 % natural sea water. Although *Ectocarpus* is an intertidal species it would not grow in such a wide range of salinities. Whitaker & Clancy (1937) found that the eggs of a species of *Fucus* would germinate in salinities ranging from 30 to 180 % sea water, with a marked optimum at 90–100 %. Their experiments were carried out at 15° C and agree quite well with the salinity toleration of *Ectocarpus* at this temperature. It is of interest to note that *Ectocarpus* will tolerate lower salinities at low temperatures and this probably accounts for the growth of species of *Ectocarpus* in salinities of 5·5 ‰ in the Baltic near Helsinki. The findings that the optimum salinity for the growth of *Ectocarpus* at 20° C was slightly above the salinity of natural sea water probably, in part, explains the poor growth of this alga in the low salinity media of Droop. As was shown experimentally, when the salinity of this medium was doubled there was a considerable improvement in growth.

The optimum pH for the growth of *Ectocarpus* was 8·0, but it grew quite well in pH's ranging from 9·0 to 7·0.

Light intensity did not have any marked effect on the growth of *Ectocarpus*. This probably indicated that the growth process of the alga was light saturated at intensities as low as 125 ft. candles (1350 lux) and that light intensities as high as 1500 ft. candles (16000 lux) did not have any inhibitory effect. However, the length of the lag phase appeared to be very markedly affected by light intensity and there was a marked optimum at 600–700 ft. candles (6500–7500 lux). The only observable change during the lag phase was the production of plurilocular sporangia followed by the release and germination of the spores. It therefore appears that light intensity has a marked effect on the production of the plurilocular sporangia or on the subsequent germination of the spores.

Temperature had much the same effect as light intensity on the growth of *Ectocarpus* in that the main effect was on the length of the lag phase and there was no marked effect on the rate of growth. *Ectocarpus* would grow at temperatures between 10 and 25° C but not at 30° C. The optimum temperature was between 15 and 20° C. In rock pools *Ectocarpus* must be subjected to temperatures much higher than this and at first it appeared strange that it would not grow at 30° C. However, it must be remembered that in rock pools the alga is not likely to be subjected to these high temperatures for long, for even if the pool temperature rises considerably during low water it will be cooled again by the incoming tide in a few hours. Ryther (1954) found that marine phytoplankton will tolerate temperatures between 10 and 30° C and the optimum was 15–25° C. Similar temperature ranges have been found for planktonic algae by Kain & Fogg (1958*a*, *b*). Hyde (1938) found that the assimilation rate of *Fucus serratus* increased with increasing temperature up to 25° C and then fell off sharply at temperatures higher than this.

In common with the findings of many other workers with a wide range of algae, the growth of *Ectocarpus* in natural sea water was stimulated by the addition of soil extract (Harvey, 1939; Pringsheim, 1946, 1951; Provasoli *et al.* 1957). Vitamin B_{12} also showed some stimulation and similar effects have been shown for a number of marine algae (Droop, 1957*b*). Although *Ectocarpus* remained viable for long periods in the dark it would not grow on organic carbon sources in the dark and is therefore probably an obligate phototroph (Fogg, 1953) although experiments have not yet been exhaustive enough to prove this.

Ectocarpus grew in several artificial sea waters but in no case was the yield greater than two-thirds that in natural sea water. Probably the addition of soil extract would have increased the yield but as the aim was to use a medium of known chemical composition this was not desirable. It was interesting to find that the low-salinity media which have been widely used for culturing

many unicellular algae were unsuitable for *Ectocarpus*. The artificial media did not appear to suffer from an excess or deficiency of trace elements and although the addition of vitamin B_{12} and liver extract showed some improvement this was not very marked. The pH of the artificial media was always adjusted to 7·5–8·0 and it is difficult to see why growth was not as good in the artificial media as in natural sea water. This is a problem that remains for further investigation.

I wish to thank Prof. G. E. Fogg for his guidance and encouragement during all stages of this investigation. This work was carried out during the tenure of a Fishery Research Training Grant for which I am indebted to the Development Commission.

SUMMARY

The growth and nutrition of *Ectocarpus confervoides* has been studied in bacteria-free cultures. Growth was estimated by dry weights determined under controlled conditions. A method for inoculation of cultures of filamentous algae is described and a table of culture media is given. Following a lag of 5–7 days there was a period of growth, which was not truly exponential, extending over a period of 35 days. Calculation of the relative growth constant for the early stages of growth indicated that the alga doubled its dry weight every 6–7 days. Cultures of *Ectocarpus* remained viable for over 1 year in the light and for over 100 days in the dark. In natural sea-water media the maximum growth was brought about by the addition of 0·5 mM potassium nitrate and 0·1 mM potassium phosphate. Ferric chloride had no effect on growth but additions of manganese chloride did cause some stimulation. Additions of some complex organic mixtures slightly increased growth but *Ectocarpus* was unable to grow on a range of organic carbon source in the dark. The optimum salinity at 20° C was somewhat higher than that of natural sea water but was apparently lower at 15° C. The optimum pH was 8·0. Light intensities between 1350 and 16,000 lux had no marked effects on the growth rate but did effect the lag, the optimum for this being 7000 lux. Temperature also had no effect on the growth rate but did effect the lag. The optimum was 15–20° C but there was growth between 10 and 25° C. A considerable range of artificial sea waters, with and without organic additions was investigated, in no case did they give a yield greater than two-thirds that in natural sea-water media. These results are discussed in relation to other workers findings.

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THE SURVIVAL OF LARVAL FISH IN THE NORTHERN NORTH SEA ACCORDING TO THE QUALITY OF THE SEA WATER

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(Text-fig. 1)

For a number of years now the presence of various species of Chaetognatha have been used to indicate sea waters of different qualities resulting from the mixing of water masses of different origins. In the North Sea and English Channel the two most important species are *Sagitta elegans* in water of mixed oceanic and coastal origin, and *S. setosa* in the indigenous water.

Russell in his series of papers (1930–47) and Corbin (1948) showed that in the English Channel more young fish were taken in the plankton collections in years when *S. elegans* rather than *S. setosa* was predominant. Wilson (1951) showed that certain invertebrate larvae thrived in 'elegans' water better than in 'setosa' water and that this could be associated with some positive beneficial factor in the 'elegans' water rather than something toxic in the 'setosa' water.

In the northern North Sea Fraser (1937) showed that the mixed conditions associated with *S. elegans* gave rise to a richer zooplankton than could be associated with either oceanic or neritic water, and Saville (1959) demonstrated the association of such mixed conditions with the success of haddock broods.

This paper is intended to show that the analysis of this association of successful survival of fish larvae with the mixed conditions typified by the presence of *S. elegans* can be taken further.

THE NORTHERN NORTH SEA

During the years 1946 to 1950 *S. setosa* was sufficiently abundant in the northern North Sea for a comparison to be made between the numbers of fish eggs and larvae found in waters where *S. setosa* and *S. elegans* respectively was present. The numbers of fish eggs and fish larvae taken by the Scottish research vessels were divided into four columns according to the *Sagitta* content of the plankton as shown in Table 1. The fish numbers for each month were separately totalled and divided to give the average per haul per month, and the twelve numbers—or less if some months contained no samples—thus obtained were averaged to give the yearly average value given in the table. No very precise comparisons are possible as cruises varied from

year to year, but such a wide system of averages should smooth out any serious discrepancies due to this factor, and give results which are comparable in a broad sense.

The table illustrates the general lack of young fish (except mackerel) in areas where *S. setosa* was the only chaetognath, and a relative abundance in areas where *S. elegans* was characteristic—see columns IV and VI. The numbers in column IV are, in effect, the demersal fish in general, and when both species of *Sagitta* are present they are seen to be about half way between those with *S. elegans* or *S. setosa* only. Mackerel larvae were abundant only in one year of this series —1950—and in 'setosa' water, so that the high figure of 144 in column III is enough to bring the 'S' average to 34·4 which is greater than the 'E' average of 28·6. Removing the weighting by mackerel makes little difference to the years 1946–9, but considerably affects 1950 so that the average 'S' number in column IV of 9·4 is much less than the 'E' number of 28·2. Column V excludes gobies, sandeels, *Gadus esmarkii* and other non-marketable species. For clupeoids the figure when both species were present is about the same as when *S. elegans* alone occurred and considerably higher than when *S. setosa* alone was recorded. This suggests, in agreement with Wilson, that no harmful factor exists with *S. setosa*—indeed an occasional 'setosa' sample is found with very high numbers of larvae—but that the mixed condition associated with *S. elegans* has a positive beneficial factor. However, this may be, that it is the survival rather than the spawning of the fish which is affected is shown by the numbers given for planktonic fish eggs which do not indicate that spawning fish show any preference for 'elegans' water, but rather the reverse.

From 1951 to 1956 *S. setosa* was not sufficiently abundant in the northern part of the North Sea for comparisons to continue on the same basis, but the collections when *S. elegans* was absent were sufficiently widespread and numerous for a comparison to be made, using only presence or absence of *S. elegans*. Absence may, of course, be due to either neritic or oceanic conditions or merely to the chance absence in the plankton sample of any specimens. Table 2 contains the results obtained from the years 1946 to 1956 inclusive, and it shows the same tendencies as those shown in Table 1, i.e. eggs more numerous where *S. elegans* was absent, but larvae more numerous where it was present.

It is realized that, in so far as the smaller the sample the less would be the chance of finding *S. elegans* and the smaller would be the numbers of fish caught, the simple division into presence or absence of *S. elegans* could result in a spurious correlation between the number of young fish and the *Sagitta*. However, the same collections treated the same way show the opposite relationship for eggs (column VIII), and this table shows much the same characteristics as Table 1 where the data are not subject to this criticism. Table 2 does, therefore, appear to have significance.

TABLE 1. THE RELATIONSHIP BETWEEN *SAGITTA ELEGANS* AND *S. SETOSA* AND THE NUMBERS OF YOUNG FISH IN THE PLANKTON COLLECTIONS

I	II				III				IV				V				VI				VII			
Year	Number of hauls				Young fish (less Clupeoids)				Young fish (less Clupeoids) and mackerel)				Commercial demersal species only				Clupeoids				Planktonic fish eggs			
	E	E+S	S	O	E	E+S	S	O	E	E+S	S	O	E	E+S	S	O	E	E+S	S	O	E	E+S	S	O
1946	96	24	19	44	16	15	2·9	3·3	16	15	2·9	3·3	3·9	2·9	1·1	1·5	41	38	0·1	0·9	No Data			
1947	117	78	71	139	25	13	5·2	19	25	12	5·0	19	7·8	5·0	1·5	9·6	18	14	2·6	77	11	2·9	3·1	3·5
1948	180	51	34	243	27	22	12	11	27	22	12	11	8·9	5·2	5·9	3·5	15	37	0·8	4·5	22	103	117	244
1949	333	94	64	75	26	12	8·1	14	25	11	4·9	14	8·5	4·4	2·0	8·1	12	5·3	0·7	1·8	54	64	115	34
1950	85	35	6	39	49	37	144	20	48	22	22	9	18	4	0·1	6	17	18	0·3	0·3	71	119	117	93
Average	—	—	—	—	28·6	19·8	34·4	13·5	28·2	16·4	9·4	11·3	9·4	4·3	2·1	5·7	20·4	22·5	0·9	16·9	39·5	72·2	88·0	93·6

E = *S. elegans* present, S = *S. setosa* present, E+S = both species present, O = neither species present. (The figures are averages per haul per month per year (see text) to smooth the differences between cruises which are not strictly comparable, year by year).

TABLE 2. THE RELATIONSHIP BETWEEN THE PRESENCE OR ABSENCE OF *SAGITTA ELEGANS* AND THE NUMBER OF YOUNG FISH IN THE PLANKTON COLLECTIONS

I	II		III		IV		V		VI		VII		VIII	
Year	No. of hauls		Young fish (less Clupeoids)		Young fish (less Clupeoids and mackerel)		Commercial demersal species only		Clupeoids		Non-commercial species only		Planktonic fish eggs	
	E	O	E	O	E	O	E	O	E	O	E	O	E	O
1946	120	63	14·9	3·7	14·9	3·7	2·7	1·6	37·8	0·6	12·2	2·1	No data	
1947	195	210	20·0	9·1	19·9	9·0	6·8	4·2	18·9	16·4	13·1	4·8	10·8	4·0
1948	231	277	23·2	12·1	23·1	12·1	8·4	3·3	20·7	4·1	14·7	8·8	34·4	188·1
1949	327	139	20·5	12·8	20·1	11·2	7·1	4·8	10·8	1·7	13·0	7·4	58·4	80·4
1950	120	45	49·5	39·9	48·1	12·3	16·2	4·7	12·6	0·3	31·9	7·6	85·0	105·1
1951	192	13	14·9	5·9	12·1	5·6	3·6	1·3	10·4	0·3	8·5	4·3	87·0	135·7
1952	388	32	11·8	4·9	11·8	4·9	3·4	1·3	19·0	0·3	8·4	3·6	87·6	207·1
1953	296	26	25·2	1·0	23·0	1·0	13·6	0·3	15·1	1·2	9·4	0·7	72·6	310·9
1954	167	53	16·0	21·0	8·5	2·3	6·8	1·1	6·5	0·1	1·7	1·2	316·1	254·7
1955	275	24	18·8	9·2	14·1	9·2	6·2	4·3	4·7	0·4	7·9	4·9	50·3	167·8
1956	235	9	20·4	2·0	20·4	1·7	5·4	0	13·6	6·0	15·0	1·7	65·7	0
Average	—	—	21·4	11·1	19·6	6·6	7·3	2·4	15·5	2·9	12·3	4·3	78·9	132·2

E = *S. elegans* present, O = *S. elegans* absent. (As in Table 1 the figures are averages per haul per month per year).

In columns IV to VII of Table 2 none of the possible forty-four pairs of numbers show the presence of more larvae when *S. elegans* was absent although, as would be expected, a small number of the individual samples contributing to these averages do show it.

It will be noted that in comparing the data in Table 1 with the first 5 years in Table 2 there are apparent discrepancies in the numbers given. This is due to the system of averaging adopted as explained above. For example, E plus E + S in Table 1 does not equal E in Table 2 because the numbers of individual samples in each category vary in each month as well as in the yearly average.

If the data are grouped by months instead of years, to show seasonal instead of annual variations, the same type of relationship is shown. The numbers for individual species of fish or fish eggs are inadequate to make a comprehensive specific survey, but it has been possible to treat some of the commoner species separately this way; the results are shown in Table 3 where the numbers are divided, as in Table 2, according to presence or absence of *S. elegans*.

The early stages of the eggs of cod and haddock are not easily separated and they are grouped together in the table as cod/haddock (column II). The later stages, after development of the pigment in the embryo, are shown in separate columns (columns III and IV). From these columns it can be seen that the early stages were sometimes more abundant when *S. elegans* was absent, but the later stages, although less numerous than the early stages, were more abundant when it was present. Eggs of whiting and plaice, both fish of a more southerly distribution than cod and haddock were overall more abundant where *S. elegans* was absent, but for long rough dab, a more northerly species in the North Sea, the differences are negligible.

All species of fish larva examined, including whiting and plaice, show a greater average abundance over the eleven year period in the presence of *S. elegans*, and although there are five pairs of monthly figures in the table (one for each of cod, lemon sole and long rough dab and two for 'other flat fish') where the opposite is shown—as against sixty-four equal or greater—the difference in each of these instances is less than 0·1.

THE ENGLISH CHANNEL

Russell in his work in the English Channel, already referred to, did not publish his data on fish eggs, which were not as full as those on the young fish, but he has kindly put them at my disposal. The number of samples per month varied from nil to five and the range in any one month was at times very high (80–7140 in February 1931), high numbers usually being of sprat eggs. Average numbers are perhaps not therefore very reliable but they are given in Table 4.

These numbers can be added to Russell's pictorial representation of the

TABLE 3. THE RELATIONSHIP BETWEEN VARIOUS SPECIES OF FISH EGGS AND PLANKTONIC FISH AND THE PRESENCE OR ABSENCE OF *SAGITTA ELEGANS* IN THE PLANKTON COLLECTIONS

EGGS

	I No. of samples		II Cod/haddock (early stages)		III Cod (late stages)		IV Haddock (late stages)		V Whiting		VI Plaice		VII L.R.D.*	
	E	O	E	O	E	O	E	O	E	O	E	O	E	O
Jan.	3	1	2·25	4·50	0·08	0	0·40	0·33	0	0	2·37	3·80	0	0
Feb.	4	2	5·83	3·00	0·08	0	0	0	0·23	0	3·73	40·50	0·38	0
Mar.	6	6	67·80	19·61	4·63	0·79	2·68	0·88	3·42	5·83	3·93	8·03	1·30	25·42
Apr.	7	4	57·41	139·48	4·36	0·33	10·17	7·30	5·64	19·80	8·75	0·23	36·70	31·80
May	5	5	7·98	34·60	0·46	0·10	2·12	0·32	3·40	4·98	0·04	0	0·22	2·06
June	2	1	5·40	0·60	0·25	0	0·25	0	3·00	8·80	0·23	0	0	0
July	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aug.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sept.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Oct.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nov.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dec.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	6	6	146·67	201·79	9·86	1·12	15·62	8·83	15·69	39·41	19·05	52·56	56·61	59·28
Av.	—	—	24·45	33·63	1·64	0·19	2·60	1·47	2·61	6·57	3·17	8·76	9·44	9·88

LARVAE

	VIII No. of samples		IX Cod		X Haddock		XI Whiting		XII Plaice		XIII Lemon sole		XIV L.R.D.*		XV *Gadus esmarkii*		XVI Other gadoids		XVII Other flat fish	
	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O
Jan.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Feb.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mar.	6	6	0·39	0·01	0·06	0·02	0·05	0·05	0·05	0	0·01	0	0·13	0·22	0·89	0·01	15·6	5·78	0	0·01
Apr.	9	6	0·91	0·27	1·58	0·47	0·38	0·05	0·52	0·14	0·02	0	2·38	0·17	5·05	1·03	11·31	3·97	0·33	0·24
May	7	7	1·64	0·90	5·10	1·16	3·54	2·07	0·76	0·12	0·35	0·41	3·27	0·54	7·00	0·91	5·84	2·26	2·24	0·59
June	9	8	0·20	0·01	1·70	0·64	6·77	3·08	0·24	0·06	3·93	1·15	0·67	0·33	0·82	0·20	2·23	0·61	4·37	0·98
July	8	8	0·03	0	0·31	0·23	3·24	2·14	0·57	0·01	3·49	0·65	0·39	0·06	0·09	0·03	1·00	0·45	5·11	0·97
Aug.	9	8	0·01	0·06	0·01	0	0·19	0·01	0·01	0·01	2·69	0·72	0·08	0·05	0	0	0·14	0·01	1·68	0·44
Sept.	6	5	0·02	0	0	0	0·06	0·06	0·02	0	2·25	0·80	0·21	0	0	0	0·07	0·06	1·63	0·64
Oct.	9	7	0·01	0	0	0	0·02	0	0	0	7·05	1·50	0·15	0	0	0	0	0	1·01	0·20
Nov.	8	2	0	0	0	0	0	0	0	0	0·84	0	0	0	0	0	0	0	0·15	0·20
Dec.	7	3	0	0	0	0	0	0	0	0	0·62	0	0	0	0	0	0	0	0·22	0
Total	10	10	3·21	1·25	9·26	2·52	14·25	7·46	2·17	0·34	21·30	5·23	7·28	1·37	13·84	2·18	36·75	13·14	16·76	4·27
Av.	—	—	0·32	0·13	0·92	0·25	1·43	0·75	0·22	0·03	2·13	0·52	0·73	0·14	1·38	0·22	3·68	1·31	1·68	0·43

E = *S. elegans* present; O = *S. elegans* absent.

* L.R.D. = long rough dab (*D. platessoides*).

TABLE 4. THE NUMBER OF FISH EGGS (EXCLUDING PILCHARD) TAKEN IN ½H HAULS WITH THE 2 M RING TRAWL IN THE ENGLISH CHANNEL (FROM UNPUBLISHED DATA KINDLY SUPPLIED BY DR F. S. RUSSELL)

	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	Average
Jan.	—	680	—	1165	Many	Many	345	71	149	149	427
Feb.	73	3290	—	1320	Many	Very many	1617	310	1223	2081	1418
Mar.	3723	—	—	2240	—	Many	540	768	627	1292	1532
Apr.	2176	560	—	—	—	—	—	630	348	540	851
Total	5972	5430	—	4725	—	—	2502	1779	2347	4062	4228
Add average of missing months	427	1523	—	851	—	—	851	—	—	—	—
Total	6399	6062	—	5576	—	—	3353	1779	2347	4062	—
Average	1562	1516	—	1394	Many	Many	838	445	587	1016	1057

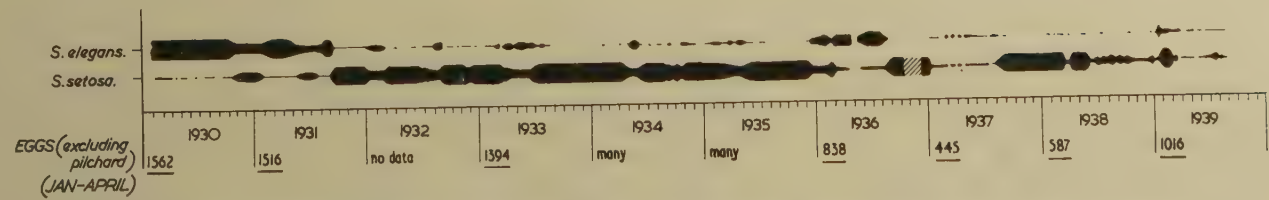

Fig. 1. The occurrence of *Sagitta elegans* and *S. setosa* off Plymouth, 1930–9 (from Russell), and the average numbers of fish eggs (excluding pilchard) taken in the same years.

abundance of *S. elegans* and *S. setosa*, taken from his papers, and reproduced here as Fig. 1. The main features revealed in this figure are: (1) the high egg numbers of 1930 and 1931 associated with *S. elegans*; (2) the rise in 1939 also associated with a small rise in *S. elegans*; (3) the increase of *S. elegans* in 1936 associated with only a poor or average egg content; (4) the high egg figures of 1933 and the 'many' eggs of 1934 and 1935 associated with *S. setosa*; (5) the poorest egg figure (1937) associated with neither and such slight rise as there is to 1938 associated with *S. setosa*. The number of eggs in the English Channel did not therefore follow the same significant pattern in relation to *S. elegans* and *S. setosa* as the larvae. There is, however, a suggestion that the small numbers of young fish after the change in *Sagitta* contributed to a shortage of eggs in later years, after they had matured.

DISCUSSION

The data from the English Channel are not directly comparable with the Scottish data presented here. The Channel data are arranged according to years when one species of *Sagitta* or the other was dominant, and the data from the whole area could thus be compared with the changing water conditions there. They show that in the years when mixed water dominated there were more young fish in the plankton, but the fish eggs were not so directly related. The Scottish data are arranged so that the area investigated is continuously subdivided into sampling localities according to the species of *Sagitta* present there. The samples were not deliberately selected or collected to test associations of this kind, but were taken incidentally to other investigations. They do strongly suggest, as did Russell's results, the association of better survival of fish larvae with *S. elegans* and mixed water than when *S. elegans* is absent. On the other hand there appears to be no such beneficial association with the production of fish eggs. There is no suggestion, of course, that the presence of *S. elegans* has itself any beneficial effect; indeed as it is a voracious predator the opposite could be assumed.

To explain this better survival we can postulate an increased productivity due to mixing water masses, each with a different limiting factor, and the increased phytoplankton production would lead to a better food supply for the young fish. Huntsman (1920) put forward evidence to show that the 'wrong ' type of water might prevent the development of fish eggs and larvae in the Atlantic near Cape Breton. The effect of different water masses on fish larvae is apparent also under aquarium conditions, and Morris (1956), for example, stated that the more intimate the association of a fish with a particular environment the more difficult it was to rear. A hint, and it is only a hint, that the difference between the survival rate of fish in one water or another is more than merely food supply is given in figures for the eggs of cod and haddock, where a differential survival is indicated before hatching and

becoming dependent on an outside source of food. This is put forward here without any experimental evidence to support it, but in view of the gradually accumulating evidence of the effects of metabolites on a wide range of marine biological processes it does seem to be a likely possibility.

SUMMARY

The numbers of fish eggs and planktonic stages of fish taken during a 5-year period by the Scottish research vessels in the northern North Sea have been associated with the presence of *Sagitta elegans* and *S. setosa* in the same collections and over an 11-year period with the presence or absence of *S. elegans*. The results confirm Russell's findings in the English Channel that fish larvae are more abundant when *S. elegans* is present than when *S. setosa* is present.

The total numbers of fish eggs do not show these differences; spawning does not appear to be any more productive in water associated with *S. elegans*, rather the reverse, and this is shown to be so for the eggs of those species separately examined. Numbers of the larvae were distinctly greater in the 'mixed' conditions indicated by the presence of *S. elegans* and a more abundant food supply is a possible cause. That the differences may signify a more fundamental effect, however, is suggested by the figures for cod and haddock eggs, which show the earliest stages to be more abundant where *S. elegans* was absent although later stages were more abundant when *S. elegans* was present.

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ON THE PIGMENT OF *IANTHINA JANTHINA* L.

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(Text-fig. 1)

The purple secretion of *Ianthina* contains one of the most striking molluscan pigments; but it appears to have escaped recent study, probably because of its instability in solution and the unpredictability of *Ianthina* strandings on coasts within easy reach of a biochemical laboratory. The main properties of this pigment were well described by Moseley (1877) from 'Challenger' material. Verne (1926) and several older authors assume that it is identical with *Murex* purple (6-6′-di-bromoindigotin), to which it bears no resemblance, either in spectral absorption or solubilities, as Moseley's observations had already shown. Nor is it identical with the blue pigment of *Velella*, on which *Ianthina* is said to feed (Kropp, 1931). More recent reviews of pigments (Lederer, 1940; Comfort, 1951) and on the biology of *Ianthina* (Fraenkel, 1927; Laursen, 1953; Wilson & Wilson, 1956) give no further information.

Moseley's observations on the fresh material suggest certain affinities to aplysiopurpurin, which is known to be a mixture of bilidiene chromoproteins (Fontaine & Raffy, 1936; Lederer & Huttrer, 1939). The stranding of a large number of *Ianthina* on the Irish coast north of Sligo in August 1960 made it possible to examine this possibility and to confirm Moseley's observations spectrophotometrically. A good deal of material was lost in transit, but since a means of handling it has now been found it should be possible in the future to transport the pigment in quantity sufficient to settle its nature.

OBSERVATIONS

The strandings of *Ianthina* took place in the third week of August 1960, after westerly winds had been blowing—probably on the 17th and 18th, when fifty-three living specimens with floats were picked up on two westerly-facing beaches, Ballineden strand near Raghly, north of Sligo Bay, and Trawalua strand on the west side of Mullaghmore, near the meeting point of Counties Sligo, Leitrim and Donegal. They were accompanied by large numbers of *Velella* and by other long-rage drift. Over the following days thirty-five shells were found on these and adjacent beaches, but others had been blown away before all the accessible strands had been examined. The sample was very like that described and figured by Wilson & Wilson (1956) with the same variety of shell form, the largest measuring 23 × 27 mm; several of the living *Ianthina*

carried attached *Lepas* and *Laomedea* like those depicted in Wilson & Wilson's plate.

In the absence of reagents, as much *Ianthina* 'ink' as possible was adsorbed on cellulose paper handkerchiefs and partially sun-dried. The live specimens were then packed in cellulose paper saturated with sea water and sent to University College by express air mail, with instructions that if no longer alive on arrival they were to be put in the refrigerator and all wrappings preserved. No *Ianthina* survived the journey and many were already decomposed, but a substantial amount of pigment had been absorbed by the packing material.

Extracts were made of this, of whole bodies eluted from the shells, and of anterior body portions where these could be separated from the remains of the visceral hump. Of these, the first were by far the most satisfactory and contained the pigment in a nearly pure state. A small amount was recovered from body extracts in acetone and separated by partition from a tomato-coloured, chloroform-soluble fraction containing liver carotenoids, but pigment from this source was very difficult to separate from an oily material with similar solubilities.

The pigment-impregnated paper was first suspended in water, to recover a precipitable protein complex, but the colour was not eluted by 0·2 M sodium bicarbonate, normal saline, or distilled water, and only slightly by 0·5 M bicarbonate, and the extracted traces were not precipitated by ammonium sulphate. The filter-cake of paper fibres was accordingly extracted with acetone, which removed the pigment quantitatively, as a dichroic, permanganate-coloured solution with a strong scarlet fluorescence in daylight.

The solubilities of the purple acetone-extracted material were as described by Moseley (1877)—it was violet-red in neutral and blue in acid solution. The blue phase is readily transferred from acetone-water mixtures into ether, less readily into chloroform. The dry pigment was soluble in lower alcohols, acetone, chloroform, petrol ether and formdiethylamide with a scarlet fluorescence most marked in alcoholic solution. The fluorescence in chloroform is about equally enhanced by methanolic zinc acetate and by methanol alone. The pigment does not appear to form a zinc complex of the bilidiene type, either before or after addition of iodine, and Gmelin's reaction is equivocal—the blue acid phase fades to yellow in about 5 min. without the characteristic 'play of colour' given by bile pigments.

The spectral curves of acetone-extracted *Ianthina* pigment are given in Fig. 1. Moseley (1877) from visual measurement described bands at about λ_{max} = **5900**, 5300, 5000 Å. We found a constant main peak at λ_{max} = 6050 with an inflexion at 5500 in neutral solution: a small maximum at 5050 appeared after zinc acetate treatment and in acid chloroform. It was absent in neutral formdiethylamide but a new maximum could be detected at λ_{max} = 3600.

Chloroform solutions of the pigment were not reduced by shaking with

aqueous hydrosulphite, and only slowly by nascent hydrogen. On standing in daylight they faded to olive-green without identifiable bands or fluorescence. None of these degradation-products gave an identifiable zinc complex.

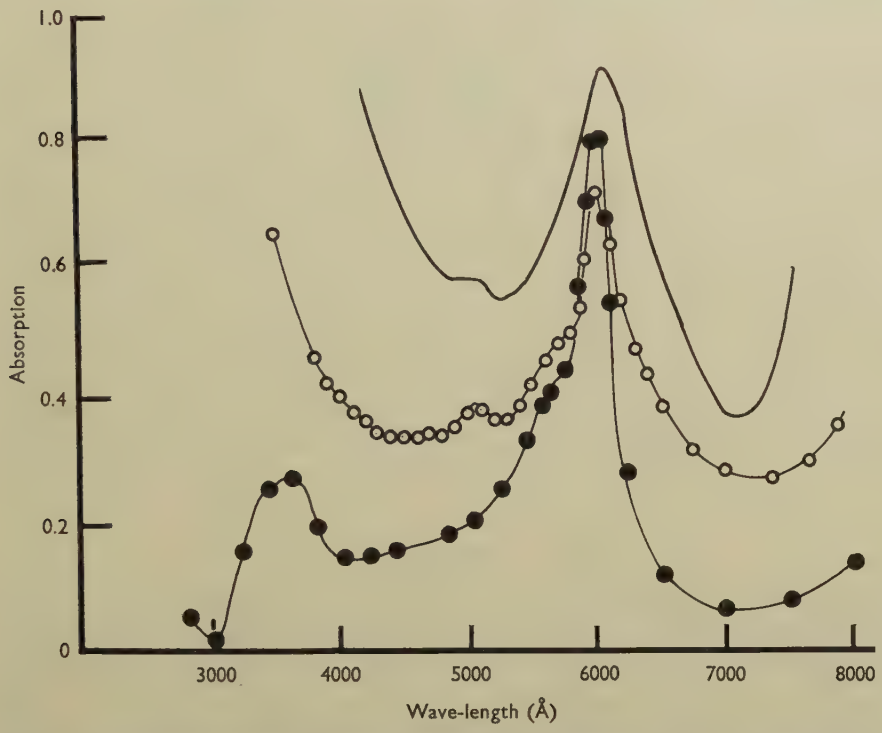

Fig. 1. Absorption spectra of *Ianthina* acetone-soluble pigment. ●—●, In neutral form-diethylamide; ○—○, in $CHCl_3$ with methanolic Zn acetate; ——, in acid $CHCl_3$.

DISCUSSION

Apart from confirming Moseley's observations, the chief conclusion is negative. The 'ink' pigment of *Ianthina* does not appear to be identical either with aplysiopurpurin or with other molluscan pigments of similar colour. A summary of those most like it is given in Table 1. The colour change and rapid extraction by acetone suggest that, as in the chromophores in *Aplysia*, it may be initially attached to protein. It fails to give the typical bilidiene reactions (Gmelin's reaction, zinc complex formation, reduction by hydrosulphite), but several other molluscan pigments which appear to be linear tetrapyrroles are atypical in one or more of these respects—thus the zinc complexes formed from haliotiviolin or its hydrochloride are non-fluorescent (Tixier & Lederer, 1949) and Gmelin's reaction is obscured by the formation of a red derivative (Comfort, 1951; Tixier, 1951). Aplysiopurpurin (Fontaine & Raffy, 1936)

TABLE 1. PROPERTIES OF VIOLET MOLLUSCAN PIGMENTS

	Spectral abs. (max.)			Zn complex			
	Acid	Neutral	Fluorescence	Spectrum (max.)	Fluorescence	Gmelin reaction	Solubility
6–6′-Dibromoindigotin	—	—	—	—	—	—	Insoluble in all ordinary solvents
'Haliotiviolin' (Tixier, 1949, 1951)	Blue: 625	Violet: 550	Nil	603	None	Red compound	Free pigment and its hydrochloride insoluble in non-polar solvents, esters soluble in ether and $CHCl_3$
'Aplysiopurpurin' (Fontaine & Raffy, 1936)	Blue	Violet: 597, 545, **493**	Red	**601**, 552, **505**	Orange	+	Prosthetic group freed by acetone is insoluble in water, petrol ether, slightly soluble ethanol, soluble $CHCl_3$, butanol
Violet component (Lederer & Huttrer, 1942)	Blue: 606, 565, **497**	Violet: (595) 538, **496**	Red	(630), **600**, 535, 505	Red	+	—
Red component	Blue: 603, 548, **498**	Red: 536, **498**	Red	**600**, 553, 507	Red	+	—
Ianthina pigment	Blue: **605**, (530), 505	Violet: **605**, 530, 360	Red++	(?) **605**, (530), 505	Red	Atypical	Soluble (from dryness) in $CHCl_3$, Et, EtOH, petrol ether

and its two main constituent pigments (Lederer & Huttrer, 1942) have a red fluorescence but differ in their spectra and solubilities from the acetone-soluble *Ianthina* pigment. The pigment spectrally closest to ianthinine is the zinc complex derived from aplysiopurpurin (Lederer & Huttrer, 1942) which is also fluorescent.

In spite of the absence of typical reactions it is tempting to infer from the similarity in band locations between ianthinine and these various bilidienes that this may be a related but atypical pigment. It differs sharply from the 'atypical' pyrrolic pigments extracted from shells in being freely soluble in organic solvents in the unesterefied state. Further work must wait until more *Ianthina* or pigment becomes available.

No pigment is extracted from *Ianthina* shells by acid. The solid spectrum of entire shells has a broadish band with a maximum falling at about 6000 Å. By dissection with 22 %, w/v, acetic acid in water a blue or violet pellicle can be separated from the shell; the bulk of the shell colour disappears, however, with the shell structure, leaving a reddish residue. It cannot therefore be assumed that the colour of the 'ink' and shell of *Ianthina* is due to the same material, since many other intense shell colours, including those of *Chlamys*, disappear in the same way under decalcification.

SUMMARY

The spectral absorption of the violet pigment extracted from dried *Ianthina* secretion by acetone is described. The colour of the secretion adsorbed on cellulose tissue is reasonably stable for purposes of transport. The pigment does not appear to be identical with the bilidiene-protein pigments of *Aplysia*, or with other violet molluscan pigments. The pigment of *Ianthina* shell is not extractable with acid, and all save that of the pellicle disappears on decalcification.

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THE BUOYANCY OF THE CUTTLEFISH, *SEPIA OFFICINALIS* (L.)

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The Plymouth Laboratory

(Plate I and Text-figs 1–12)

Cuttlefish are amongst the most remarkable of all animals, and we can well understand why Paul Bert (1867) was inspired to undertake a general study of *Sepia officinalis* L. This he vowed not to leave until the physiology of *Sepia* was as well understood as that of the frog. Bert did little work on the cuttlebone; he analysed the gas contained in it and expressed the opinion that this gas would vary according to circumstances, in the same way as the gas in the swim bladder of fish, then the subject of the beautiful experiments of Armand Moreau (1876). The lack of interest of physiologists in the cuttlebone, one of the most common objects on our sea shore, is perhaps excusable, for the cuttlebone is dead. In this and the following three papers we show that, although dead, the cuttlebone is not unchanging and that the cuttlefish can use it as a variable buoyancy tank. This extraordinary animal can change its density quickly and does so in response to changes in light intensity. Finally we show that liquid is probably moved in and out of the cuttlebone by an osmotic mechanism, and not by changes in the internal gas pressure which is the method used in the swimbladder.

The cuttlebone has a special interest in that it is closely related not only to the shells of the living *Nautilus* and *Spirula*, but also to the shells of the fossil Nautiloidea, Ammonoidea and Belemnoidea (see Naef, 1923; Morton, 1958). These animals dominated the Palaeozoic and Mesozoic seas and their evolution from a crawling to a free-swimming life was probably determined by the use of the shell as a buoyancy device. We hope in the light of new knowledge of the cuttlebone to see more clearly how these important animals lived. The chambers of the cuttlebone contain liquid as well as gas, and the cuttlefish changes its density and posture by varying the amounts of liquid which the chambers of the bone contain. If the fossil cephalopods could also have done this their behaviour must have been very different from that postulated on the usual assumption that their chambered shells were completely filled with gas.

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MATERIAL AND METHODS

Cuttlefish, *Sepia officinalis* (L.), were obtained from the trawling grounds off Plymouth, mostly in depths between 25 and 40 fathoms. The animals were brought to the Laboratory daily and were studied ashore. In March 1959 a special cruise was made aboard R.V. 'Sarsia' to study cuttlefish immediately they were brought aboard. These are referred to as freshly caught animals.

Animals were not kept longer than two weeks before being used for experiment.

Cuttlebones were dissected from *Sepia* anaesthetized with $\frac{1}{2}$% urethane in sea water. Sandoz MS 222 was tried but, although preferable in some respects, it has the disadvantage that the cuttlefish usually discharges its ink, sometimes to such a degree that the ink sac is almost emptied.

Weighings of less than 10 g were made on accurate torsion balances. Material weighed at sea was stored in sealed polythene bags in closed jars, and reweighed a few hours later ashore.

The volume and density of a cuttlebone were found by weighing it first in air and then under sea water together with enough lead to make it sink. Now, if w is the weight of the bone in air, y is the weight of the bone and the lead under sea water, and z is the weight of the lead under sea water; then the negative weight of the cuttlebone in sea water, u, equals $z-y$. According to the principle of Archimedes the loss in weight of the cuttlebone on putting it under sea water, i.e. $w+u$, is equal to the weight of the sea water displaced. If the weights are measured in grams then the volume, v, of the cuttlebone is $(w+u)/1{\cdot}026$ ml., where 1·026 is the density of the sea water.[1] The density d, of the cuttlebone is equal to w/v.

Other methods are given in the various sections below.

RESULTS

DIFFERENCES IN BUOYANCY BETWEEN DIFFERENT CUTTLEFISH

Cuttlefish kept in aquaria appear to differ greatly in buoyancy. Two groups of animals were chosen: in one group the animals appeared to be very buoyant, these we shall call 'floating' animals; in the second group the cuttlefish could rest on the bottom of their tank with no apparent effort, these we shall call 'sinking' animals. The cuttlefish were anaesthetized and then weighed both in air and under sea water. Floating animals were less dense than sea water and their weight in sea water was found by weighing them together with a known weight of lead. The cuttlebones were now dissected from the cuttlefish and their volumes, densities, and weights under

[1] During the course of the experiments the density of the sea water in the reservoirs of the laboratory varied a little from the value of 1·026 given above. The small differences in volume and density which these variations would give have been neglected in the calculations of this communication.

sea water were found. Finally the cuttlefish without their cuttlebones were weighed under sea water. Table 1 gives the weights in sea water of the intact *Sepia*, its cuttlebone, and the animal without its cuttlebone, as percentages of the weight of the whole *Sepia* in air. It can be seen that over 80% of the difference in density between the 'floating' and 'sinking' cuttlefish is attributable to differences between their cuttlebones. This is probably an underestimate since, as was later found, cuttlebones of low density take up sea water quicker than cuttlebones of high density and thus will be more changed during the measurements under sea water.

TABLE 1. WEIGHTS IN SEA WATER OF DIFFERENT PARTS OF CUTTLEFISH AS PERCENTAGES OF THE WEIGHT OF THE WHOLE ANIMAL IN AIR

	Whole animal (%)	Mean (%)	Bone only (%)	Mean (%)	Animal minus cuttlebone (%)	Mean (%)
'Floating' animals	−0·98 −0·93 −0·71	−0·87	−4·50 −4·81 −4·28	−4·53	+4·40 +3·88 +3·57	+3·95
'Sinking' animals	+0·35 +0·38 +0·54	+0·42	−3·69 −3·56 −3·19	−3·48	+4·04 +4·03 +3·73	+3·93
Difference between means	—	1·29	—	1·05	—	0·02

Cuttlefish which had just been trawled from about 70 m were studied aboard ship. It was found that their cuttlebones exhibited a much smaller range of densities than those caught in the same way but studied after keeping in an aquarium ashore. The freshly caught animals had cuttlebones whose densities fell within the range 0·57–0·65 (mean = 0·62, S.D. = 0·02, N = 17), whilst those studied ashore had bones whose densities varied between 0·48 and 0·71 (mean = 0·60, S.D. = 0·06, N = 38). It seemed, therefore, that the cuttlebone was not an unchanging organ but that a cuttlefish could vary the density of its cuttlebone. It is shown later that quite quick changes in cuttlebone density can occur in the living healthy cuttlefish (Denton & Gilpin-Brown, 1961*a*).

THE STRUCTURE OF CUTTLEBONE

The standard description of the cuttlebone is that of Appellöf (1893), and the following account is based on his work supplemented a little by our own observations. In a large adult the bone consists of about 100 lamellae of calcified chitin placed one above the other and held about 0·6 mm apart by numerous pillars. The spaces between the lamellae form chambers which are themselves divided by about six thin membranes running parallel to the lamellae. The chambers are sealed laterally and anteriorly by the thick calcified layer forming the curved dorsal surface of the cuttlebone. They are

laid down successively, the newer ones below the older, as the animal grows. Sections through the cuttlebone are shown in Text-fig. 8 and Pl. 1. In the region labelled *xy* the posterior margins of the lamellae turn over to close the chambers. The surface so formed has a special importance in the physiology of the cuttlebone. In *Sepia* it represents the active part of the siphuncle which in *Nautilus* and *Spirula* is a closed tube passing through the chambers of the shell. We shall refer to this surface as the 'siphuncular' surface.

THE INDEPENDENCE OF THE CHAMBERS

A chamber of the cuttlebone, with its numerous pillars and thin horizontally dividing membranes, appears to be a very complicated structure. The following experiment shows it to be functionally very simple. A needle was pushed from the ventral surface about half way through a cuttlebone thus puncturing about half of its chambers. This half-punctured bone was now placed under sea water containing *Sepia* ink in a vacuum desiccator and gas was pumped out of the desiccator with an efficient vacuum pump. When bubbles no longer came from the hole in the cuttlebone atmospheric pressure was restored and this pushed inky liquid into the space from which the gas had just been extracted. The cuttlebone was then removed from the ink, dried gently and sawn up. It was found that the ink had only penetrated into those chambers which had been punctured, but that these chambers were completely filled with ink (Pl. 1). It is clear that the chambers of the cuttlebone are independent of one another but that within any one chamber gases and liquids are fairly free to move.

THE PROPORTIONS OF GAS, LIQUID, AND SOLID IN A CUTTLEBONE

The weight, density, and volume of a freshly dissected cuttlebone were found. The cuttlebone was then pierced several times with a needle so that all its chambers were punctured. It was now placed in sea water under vacuum. When no more bubbles came from the holes in the cuttlebone atmospheric pressure was restored. Several hours later, when the spaces in the cuttlebone were completely filled with sea water, the cuttlebone was taken out of the water, dried gently on a cloth and weighed in air. Finally the bone was dried in an oven at 110° C to constant weight in air.

EXPLANATION OF PLATE I

Sepia officinalis (L.). A. Transverse section of the cuttlebone. B. Longitudinal section of the cuttlebone, showing the siphuncular wall *x–y*. *x* is posterior to *y*. A number of chambers were punctured from the ventral side of the cuttlebone, and gas was removed through the hole by vacuum. The pressure was then brought back to atmospheric, and inky water filled the chambers from which gas had been removed.

Dorsal

A

B

(*Facing p.* 322)

We shall now give a numerical example of the calculation used to derive the proportions of gas, liquid, and solid spaces in the bone.

Initial weight of bone in air	30·5 g
Initial density of bone	0·68
Initial volume of bone	45·1 ml.
Weight of bone full of sea water	55·1 g
Final dry weight of cuttlebone	17·5 g
Therefore, water lost on drying was	37·6 g

If we assume that the liquid originally in the cuttlebone had the same composition as sea water, then the cuttlebone would have contained 1·3 g of salt before drying at 110° C. The final liquid content of the bone (i.e. before drying) is thus equal to the water lost on drying plus this calculated weight of salt:

$$37{\cdot}6 + 1{\cdot}3 = 38{\cdot}9 \text{ g.}$$

Its density would have been approximately 1·026, so that its volume would have been

$$\frac{38{\cdot}9}{1{\cdot}026} = 38 \text{ ml.}$$

But this is a volume equal to that of both the liquid and the gas space which the cuttlebone initially contained and we can therefore write

$$\frac{\text{Volume of liquid and gas spaces}}{\text{Volume of bone}} = \frac{38}{45{\cdot}1} \text{ or } 84{\cdot}1\%$$

The volume of the dry matter of the cuttlebone is therefore

$$100 - 84{\cdot}1 = 15{\cdot}9\%,$$

and the weight of this dry matter is the final dry weight minus the weight of salt

$$17{\cdot}5 - 1{\cdot}3 = 16{\cdot}2 \text{ g.}$$

Now the weight of liquid which the bone initially contained is equal to the initial weight of the bone minus the weight of the dry matter

$$30{\cdot}5 - 16{\cdot}2 = 14{\cdot}3 \text{ g.}$$

If the volume of this liquid is obtained by dividing its weight by 1·026, the volume of the initial gas space can be found by difference.

To summarize, we give the approximate initial constitution of the bone per 100 ml. of bone:

	Weight (g)	Volume (ml.)
Dry matter	36	16
Liquid	32	31
Gas space	Trivial	53

The density of the dry matter is

$$\frac{36}{15{\cdot}9} = 2{\cdot}26.$$

This value is intermediate between the densities of chitin and aragonite, the substances of which the cuttlebone is largely constructed (Richards, 1951; Clarke & Wheeler, 1922; Nicol, 1960). A second cuttlebone which was studied in the same way gave results almost identical with those given above (the density of the dry matter was found to be 2·24). Many of the other experiments described later required the determination of water, dry matter and gas, and the results of some of these experiments are shown in Text-fig. 7.

The proportion of the animal which is cuttlebone is almost constant and varies little with the sex and age of the animal. It may be that baby cuttlefish and ripe females form exceptions to this rule, but neither of these two groups has been studied.

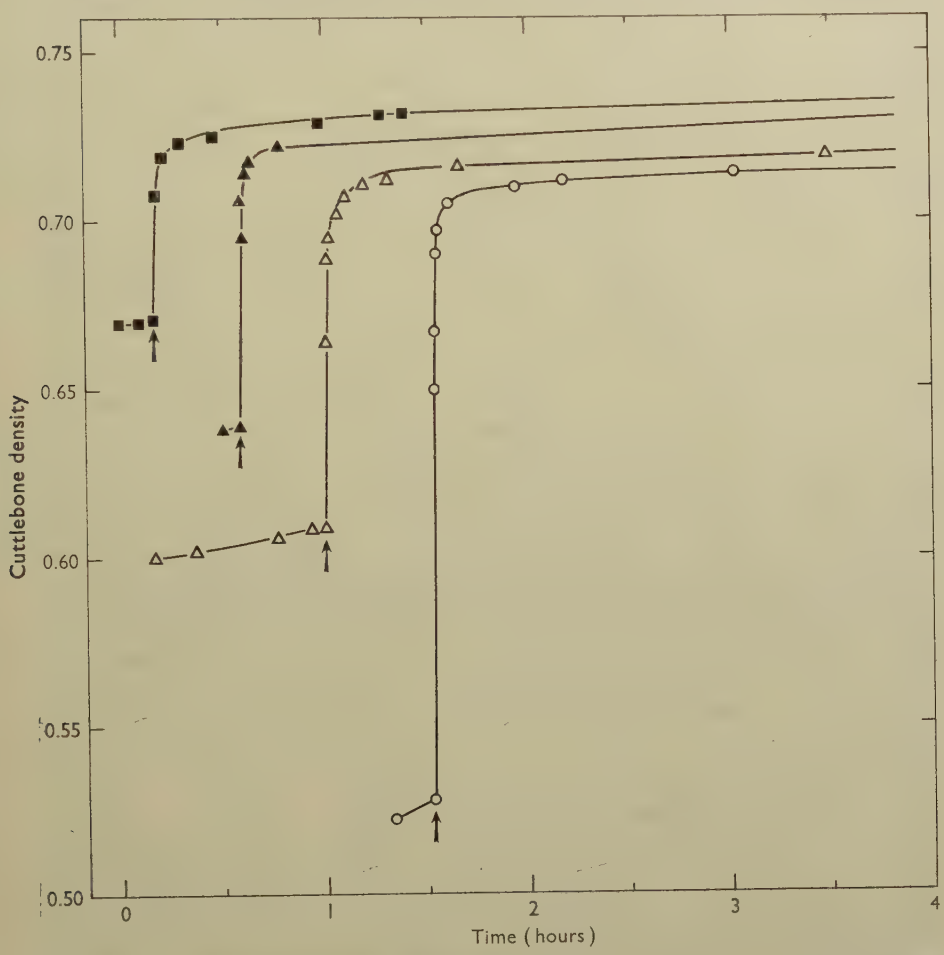
THE PRESSURE OF GAS IN THE CUTTLEBONE

When the cuttlefish is at depth in the sea, unless the hydrostatic pressure were opposed by some other force, the cuttlebone would fill with water. In the swim bladder of a fish, gas is secreted into or reabsorbed from the swimbladder and the volume of the swimbladder is held approximately constant at 5% of the fish's total volume. The pressure of gas within the swimbladder always equals the external hydrostatic pressure of the sea (Moreau, 1876; Harden Jones & Marshall, 1953). We may then ask first whether this system which works so well in the fish is also used in *Sepia*. On this hypothesis, in an animal living at 70 m the cuttlebone would contain gas at a pressure equal to the external pressure, i.e. 8 atm (7 atm for the sea and 1 for the air above it). We should expect that if an animal were freshly trawled from this depth, either that gas bubbles would be found in the tissues around the cuttlebone, or that on puncturing a stream of bubbles would come from the hole. In fact gas is never found in the tissues around the cuttlebone and, on puncturing under sea water, no stream of bubbles comes from the hole but instead the weight of the cuttlebone suddenly increases as water enters the bone. The gas within the cuttlebone, far then from being at 8 atm pressure, appears to be at a pressure less than atmospheric.

Ashore, freshly dissected cuttlebones of varying density were placed under sea water and punctured in several places so as to pierce all the chambers. Before and after puncturing such a cuttlebone it was weighed under sea water together with enough lead to make it sink. The results of this experiment are shown in Text-fig. 1 where cuttlebone density is plotted against time. It can be seen that on puncturing there is a very rapid increase in density as sea

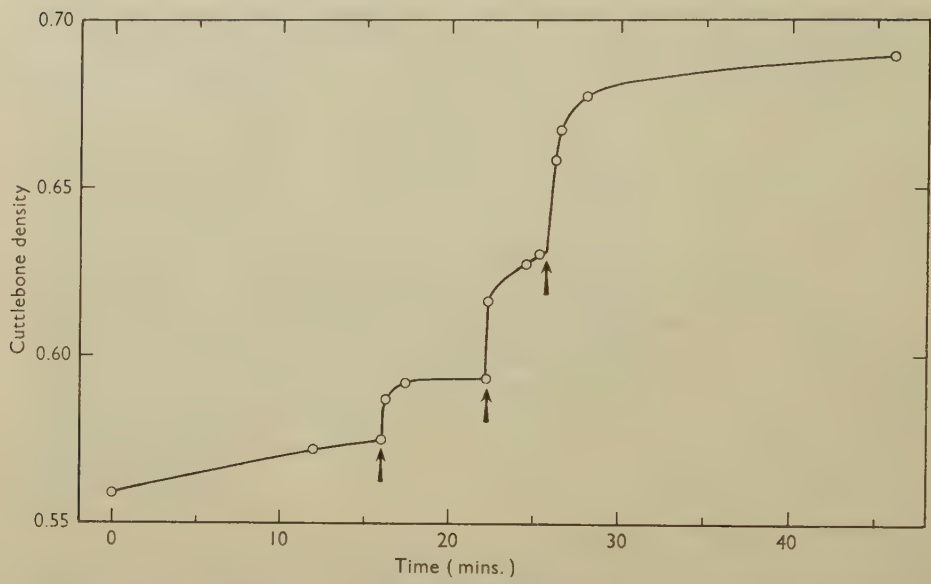
water rushes into the cuttlebone. The final density of a cuttlebone after puncturing is always very close to 0·73 no matter what its initial density.

The pressure of gas in the cuttlebone appears to be less than atmospheric throughout the larger chambers for, as a needle was pushed progressively



Text-fig. 1. Changes in density of cuttlebones of various initial densities punctured under sea water. The times of puncture are shown by the arrows. For clarity the curves are arbitrarily displaced along the abscissa.

farther into the cuttlebone, more water was taken up with each increase in the number of chambers punctured. Text-fig. 2 shows an experiment in which the larger chambers of a cuttlebone were punctured in three steps. With each increase in the number of chambers punctured there is an increase in the density of the bone.



Text-fig. 2. Change in density of a cuttlebone placed under sea water and punctured three times in such a way as to progressively pierce more chambers. The times of puncture are shown by arrows.

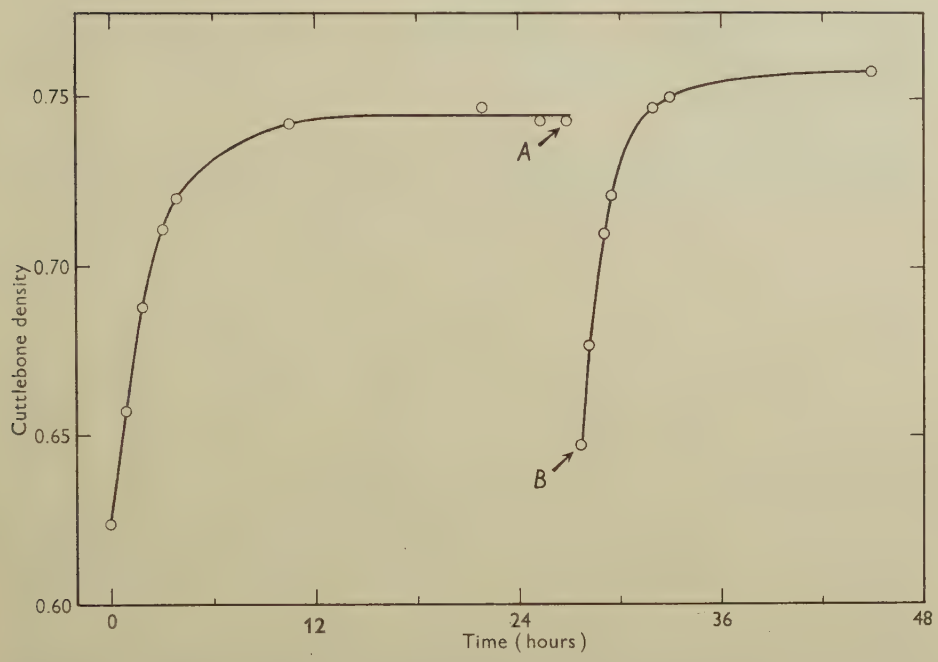
Some experiments were made to show that the rapid uptake of liquid on puncturing could not come from the solution of a very soluble gas.

(i) Text-fig. 3 shows an experiment in which a cuttlebone increased in density after being soaked in sea water for some hours. At the time *A* liquid was extracted from the cuttlebone under vacuum and then at time *B* the bone was replaced under sea water, when its density increased again. The initial rate of increase in density was almost the same as that after time *B*. This shows the initial uptake of water is unlikely to have been limited by the solution of gas.

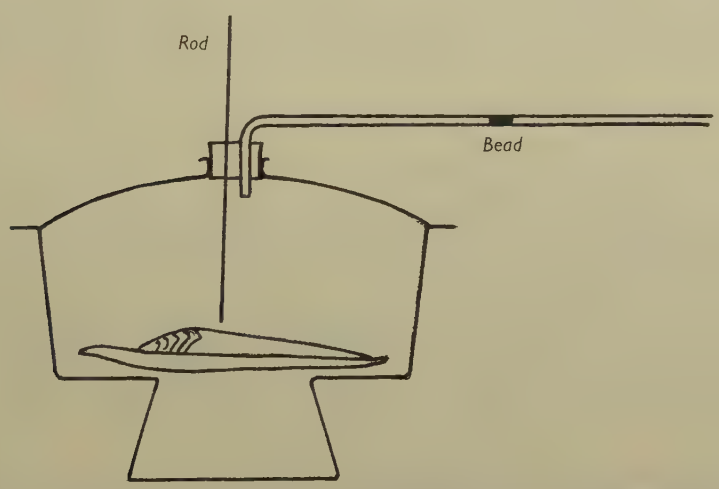
(ii) A freshly dissected cuttlebone was dried gently and placed in a vacuum desiccator closed by a rubber bung. The cuttlebone could be punctured with a steel needle passing through a small greased hole in the bung, and any volume change in the system could be measured by the movement of a bead of liquid in a horizontal tube (Text-fig. 4). On puncturing the cuttlebone the bead moved suddenly towards the desiccator, showing that a volume of *gas* had entered the cuttlebone. This volume of gas was found to be approximately equal to that volume of sea water which the cuttlebone would have taken up if it had been punctured under sea water.

(iii) As a variant on experiment (ii), cuttlebones were weighed under sea water, then punctured in air and immediately returned to sea water and re-weighed. The results of two such experiments are shown in Text-fig. 5. It can be seen that only a small uptake of sea water took place and this could be mostly accounted for by the differences between the temperature and pressure of the sea water and the air in which the cuttlebone was punctured.

(iv) A cuttlebone was soaked in sea water saturated with carbon dioxide so that the gas spaces of the cuttlebone were filled with carbon dioxide. Text-fig. 6 compares the puncturing under sea water of this cuttlebone (*B*) with a fresh cuttlebone (*A*). At the



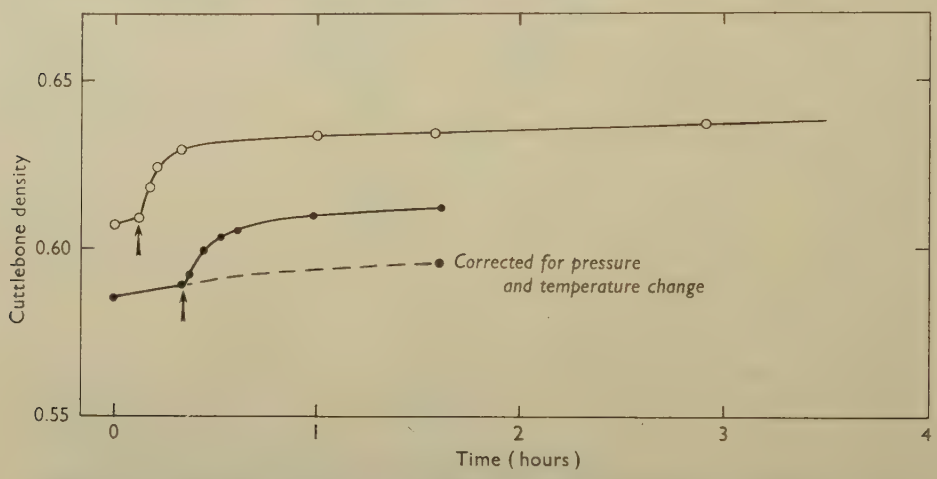
Text-fig. 3. Change in density of a cuttlebone immersed in sea water after removing its siphuncular membrane. At *A* the bone was placed under partial vacuum and liquid extracted through the siphuncular surface. At *B* atmospheric pressure was restored, the bone then took up water again at approximately the same rate as at the beginning of the experiment.



Text-fig. 4. Apparatus used to measure the uptake of air when a cuttlebone is punctured in air. The rod, which passes through a small greased hole in the rubber bung, is used to puncture the cuttlebone. The uptake of air is measured by the subsequent movement of the bead of liquid.

time of puncturing *B* the pressure of gas within the bone was greater than atmospheric and some bubbles came from the hole. It will be seen that the increase in density with the solution of the very soluble carbon dioxide is very much slower than the increase in density of the fresh cuttlebone.

The results of the four experiments just described cannot be explained on the basis of a very soluble gas, but only by the assumption that the cuttlebone contains gas under a pressure which is less than atmospheric.



Text-fig. 5. Change in density of the cuttlebone. The cuttlebones were weighed under sea water. After the second weighing they were taken into the air, dried and then punctured in several places so as to pierce all their chambers. They were then replaced in sea water and their change in weight followed. The very small changes in density can largely be accounted for by differences in pressure and temperature between the air in which the bone was punctured and the sea water in which weighings were made. The arrows show the times of puncturing.

THE COMPOSITION OF CUTTLEBONES OF VARYING DENSITY

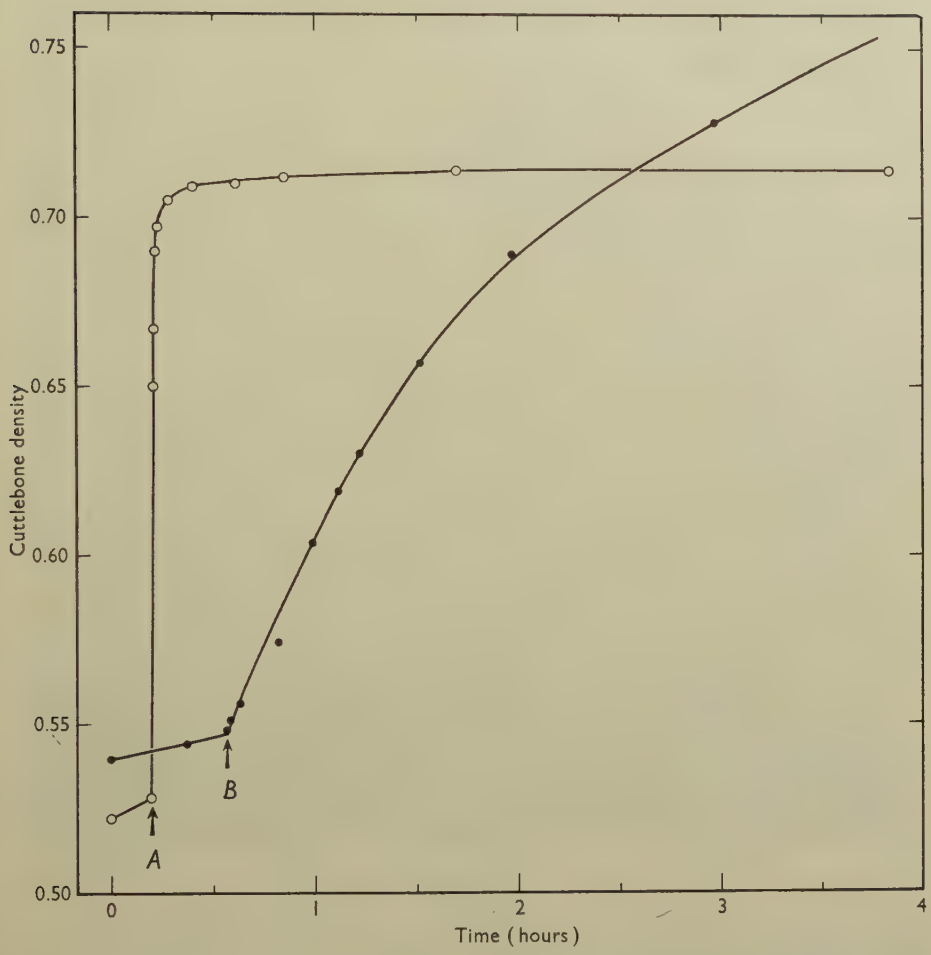
The ratio of $\frac{\text{weight of dry matter (g)}}{\text{volume of cuttlebone (ml.)}}$ was almost constant at 0·38 no matter what the density of the cuttlebone. All the density differences between different cuttlebones could be accounted for by differences in their content of liquid; thus a cuttlebone of density 0·5 contains about 10% volume of liquid, whilst a cuttlebone of density 0·7 contains about 30% of liquid.

If we take the average density of the dry matter of the cuttlebone as 2·25 (p. 324) then the constitution of a cuttlebone can be calculated as follows[1]:

The volume of dry matter per unit volume of cuttlebone is

$$\frac{\text{weight of dried cuttlebone}}{2{\cdot}25 \times \text{volume of cuttlebone}}.$$

[1] Small corrections were made for the salt content of the dried bones but, for the sake of simplicity, these are not described here.



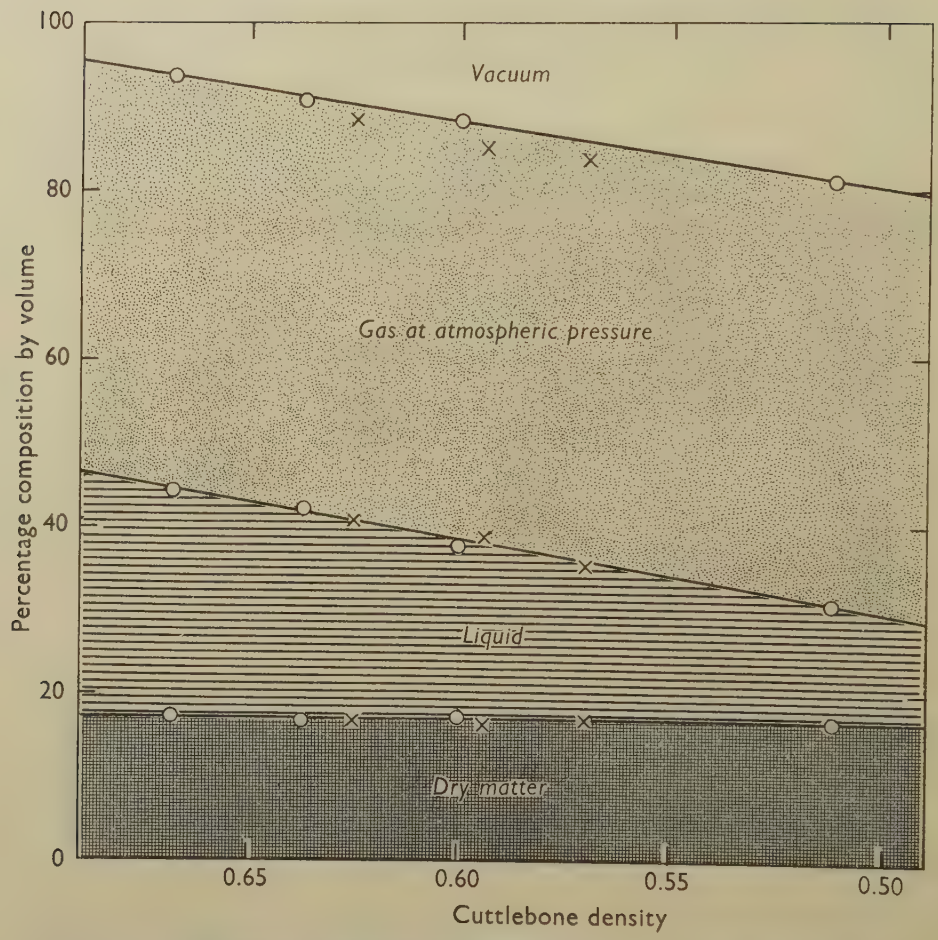
Text-fig. 6. Changes in density of cuttlebone: *A*, freshly dissected and punctured under sea water; *B*, soaked for many hours in sea water saturated with carbon dioxide, and then punctured under sea water. The arrows mark the times of puncture.

The volume of liquid per unit volume of cuttlebone is

$$\frac{\text{Initial weight of cuttlebone in air} - \text{weight of dried cuttlebone}}{\text{volume of cuttlebone}}.$$

The volume, Vg, of the gas space which the cuttlebone initially contained is

Volume of cuttlebone − (volume of dry matter + volume of liquid initially present).



Text-fig. 7. The composition of cuttlebones of different densities. If the gas within the cuttlebone is brought to a pressure of one atmosphere its volume and hence mass remains constant no matter what the density of the bone. The gas is normally at less than atmospheric pressure and fills both the volume indicated by the stippled area and also the clear space above this area. Variations in density are brought about by changing the liquid content of the bone. ○, Bones punctured under sea water (see Text-fig. 1). ×, Bones from which the siphuncular membrane was removed and which were then soaked in sea water (see Text-fig. 11).

The volume of the gas, when reduced to atmospheric pressure (at ambient temperature) is

$$Vg - \text{volume of liquid which enters on puncturing.}$$

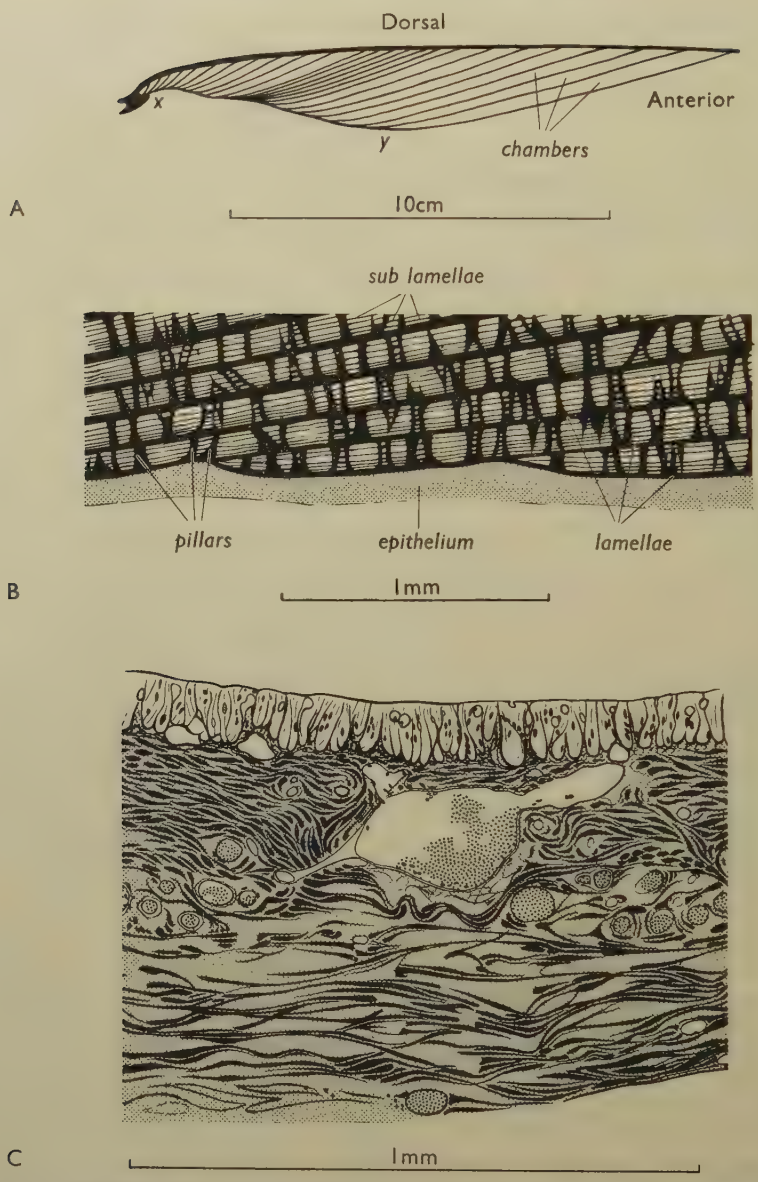
The results obtained on a number of cuttlebones are shown in Text-fig. 7. It can be seen that for all the cuttlebones the volume of gas reduced to atmospheric pressure remains almost constant. Thus the *mass* of gas within

the cuttlebone remains constant with change in cuttlebone density, and there can be no question of an evolution of gas expelling liquid to make the density of the cuttlebone fall. The fraction marked 'vacuum' (Text-fig. 7) represents the volume of liquid which enters on puncturing under sea water. We are here imagining that the 'gas' space is divided into two parts, one a vacuum and the other containing gas at atmospheric pressure. In fact, before puncturing, the gas is distributed throughout the space of a chamber of the cuttlebone at a pressure less than atmospheric. The average pressure of gas within the cuttlebone when its density is 0·62 (the average value for freshly caught *Sepia*) is about 0·83 atm.

THE SITE OF GAIN AND LOSS OF LIQUID IN THE CUTTLEBONE

The cuttlebone's dorsal surface is covered with a thick layer of calcified chitin, and the anterior ventral surface runs parallel to the many layers of calcified chitin of the lamellae. Anatomically the most likely place for liquid exchange between the cuttlebone and the rest of the animal is the siphuncular wall, *xy* of Pl. I and Text-fig. 8, which is formed by the turned over ends of the lamellae. In life this region is separated from the viscera of the cuttlefish by a thick membrane which often splits into two layers, one a yellowish layer which sticks to the siphuncular wall of the cuttlebone, and the other a fibrous layer which comes away readily from the cuttlebone. The yellowish layer we shall describe as the siphuncular membrane.

The cuttlebone contains gas and, if it is not completely impermeable, we should be able to extract some liquid merely by placing the cuttlebone under reduced pressure and allowing this gas to expand. Cuttlebones from which the yellowish membrane had been gently removed were placed ventral side uppermost in a vacuum desiccator connected to a pump and a mercury manometer. The pressure in the desiccator was reduced in small steps and the cuttlebone carefully observed. It was found that liquid does leave the cuttlebone, but only through the siphuncular wall. As the pressure is reduced drops of liquid suddenly begin to 'sweat' out of this wall. The liquid does not come out of all chambers simultaneously but usually comes first from the older and more posterior chambers; only when the pressure is further reduced does it come from the other chambers. We may give as examples two such experiments. With a cuttlebone of density 0·60, the liquid came first from the oldest and smallest chambers when the pressure was 88 mm Hg below atmospheric pressure, and from all the chambers except the four newest, when the pressure was 180 mm Hg below atmospheric pressure. The average pressure of gas predicted from Text-fig. 7 for a cuttlebone of this density is 156 mm Hg below atmospheric. In a similar experiment on a cuttlebone of density 0·56 some liquid was observed to come from the oldest chambers at 155 mm Hg below atmospheric pressure and from all chambers except the



Text-fig. 8. Structure of the cuttlebone and the siphuncular membrane. A. Diagrammatic longitudinal section through a cuttlebone from an adult animal which would have about 100 chambers. The siphuncular surface is marked *xy*. B. Detailed longitudinal section showing the siphuncular surface of a few chambers. (Simplified from a 50 μ celloidin section of decalcified bone; stain, acid fuchsin.) C. Transverse section through a part of the shell-sac lying against the siphuncular surface (*xy*) of the cuttlebone. The thin siphuncular membrane is uppermost and below this can be seen a large vein (also shown in the reconstruction of Fig. 10) whose tributaries communicate with numerous spaces just below the membrane. Many of these smaller vessels contain fixed blood (heavy stipple).

newest four at 264 mm Hg below atmospheric. The average pressure of gas predicted was 190 mm Hg below atmospheric pressure. This experiment shows that the pressure of gas within the cuttlebone is probably not uniform but differs between the different chambers. This conclusion was confirmed later by direct pressure measurements (Denton & Gilpin-Brown, 1961*b*).

The average reduction of pressure which will make liquid leave the cuttlebone is always approximately equal to the pressure difference between the cuttlebone gas (predicted from the experiments on the puncturing of cuttlebones) and atmospheric pressure. This means that surface forces (capillary attractions) do not play an important role by hindering the flow of liquid in and out of the cuttlebone. Although the pores through which liquid is moved are probably very small, the chitinous material of which the cuttlebone is partly formed is not very 'wettable'. If the pen from *Loligo*, which is made of very similar material to the chitin of the cuttlebone, is placed under sea water in a vacuum desiccator and the external pressure is reduced, gas bubbles form much more readily on the pen than on the glass walls of the desiccator and the pen periodically rises to the surface and discharges its bubbles just as does a grape in champagne (Bragg, 1925).

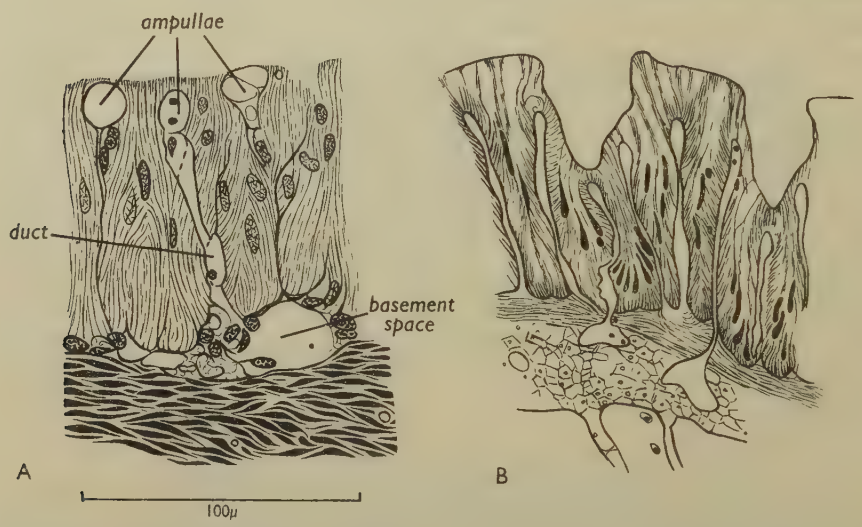
In all these experiments the newest six or so chambers never lost liquid when the pressure was reduced. The reason for this is given by Denton & Gilpin-Brown (1961*b*).

THE MICRO-ANATOMY OF THE SIPHUNCULAR WALL OF THE SHELL-SAC

The experiments described show that liquid exchange can occur across the siphuncular surface of the cuttlebone. This surface is covered by a specialized membrane, and a few square centimetres of this membrane were fixed in formalin, embedded, and sectioned at 16μ normal to the siphuncular surface. A modification of Masson's trichrome stain was tried but haematoxylin followed by Van Geison's connective tissue stain was found to be more useful. In the siphuncular region the wall of the shell-sac is very fibrous and contains numerous arteries and veins (Text-fig. 8C). The siphuncular membrane lies above a thick bed of connective tissue and is supported by a meshwork of thin connective tissue fibres at its base. Some of these fibres penetrate almost to the free surface which, in life, is applied to the siphuncular surface of the cuttlebone, (Text-fig. 9A). The cellular constituents are supported in cups of connective tissue fibres and sections made tangential to the membrane's surface show that these cups are roughly circular in cross-section.[1]

[1] According to Appellöf when a chamber is completed the epithelium which has secreted it begins to secrete the next chamber except at the siphuncular end where the epithelium is infiltrated with connective tissue from the layers lying below and the secreting cells degenerate.

The most distinctive features of the membrane are the numerous ampullae (Fig. 9A) which can be seen in the membrane, close to its free surface. These ampullae are connected by very fine ducts lying between the cups of connective tissue and leading to spaces in the basement connective tissue which in turn communicate with the veins. From a series of sections a reconstruction was made (using enlarged photographs of each section to ensure precise orientation) of all the ampullae, ducts, spaces, and veins which



Text-fig. 9. A. *Sepia officinalis*. Camera lucida drawing of a section of the siphuncular membrane showing a duct joining an ampulla with one of the spaces in the basem ent connective tissue. B. *Nautilus*. Detail of the siphuncular membrane for comparison with that of *Sepia*. Redrawn to be of approximately the same scale as A from a lithograph by Willey (1902).

were definitely inter-communicating in an area of just over half a square millimetre. The reconstruction is shown in Text-fig. 10. In the same figure, and to the same scale, this reconstruction is compared with the picture obtained by an injection of the shell-sac vessels with *Sepia* ink. The two are very similar, though the injection brings out the finer detail which could not be reliably resolved in the reconstruction. There is little doubt that the ampullae of the siphuncular membrane communicate through their ducts, with a series of spaces in the basement connective tissue; these in turn communicate with the underlying veins. Some arterioles were traced as far as the siphuncular membrane where they became much finer than the ducts, but no connexion with the ampullae could be found. Injection of the arterial system confirmed this, for the injected vessels ended in very small capillaries at the membrane.

The micro-anatomy of this region shows then a system of vessels which

can bring liquid from the circulation into close communication with the liquid within the cuttlebone. Although communicating with the blood system these ampullae and their ducts are probably not a functional part of the circulation, for fixed blood was never seen in the ampullae and their ducts even although it was present in very much smaller blood vessels in the underlying connective tissue (Text-fig. 8c).

Text-fig. 10. Above, a reconstruction of all the vessels found to communicate with the ampullae in the siphuncular membrane (here above the plane of the drawing). The basement spaces and the ducts leaving them are shown in black whilst the veins in the underlying connective tissue are shown stippled. Below, to the same scale, the appearance of similar ducts and spaces following an injection of veins with *Sepia* ink.

RESULTS WITH OTHER CEPHALOPODS

Sepia elegans

Measurements were made on two freshly caught specimens of *S. elegans* which were in very good condition. One animal was 0·38% denser than sea water, the other 0·38% less dense than sea water. The initial densities of their cuttlebones were 0·47 and 0·42 and the ratios of cuttlebone volume to *Sepia* volume were 8·4 and 7·1% respectively. On puncturing under sea water both bones took up sea water and attained the final density of 0·63. Although the bone of *S. elegans* is a little less dense than that of *S. officinalis* (it is perhaps a little less strongly constructed) these figures show the essential similarity between the cuttlebones of the two species.

An examination in the British Museum of Natural History of a collection of cuttlebones from many different species of cuttlefish showed that they differed appreciably in the ratio of siphuncular surface area to the total surface area of the cuttlebone. We should expect that the species with cuttlebones

with relatively large siphuncular surfaces are those which can change their density most quickly.

Loligo forbesi

As we have seen, the cuttlefish can vary the density of its cuttlebone but the whole animal is nevertheless always fairly neutrally buoyant. The common squid, *L. forbesi*, whose pen is without gas spaces, was also studied. Two squid weighing 620 and 280 g in air, weighed 27 and 11 g respectively in sea water. The pen of *Loligo* only amounts to about $\frac{1}{2}$% of the animal's total weight and since its density is only 1·2 it plays a negligible role in deciding the buoyancy of the animal. Thus we find that the pelagic *Loligo* is some 4% denser than sea water, whilst the bottom-dwelling *Sepia* is approximately neutrally buoyant; a fact which will not surprise us if we recall how these animals hunt their food.

Although the pen, after drying with a cloth, appears to be a dry structure it loses about half of its weight on drying in an oven at 110° C. It seems likely that the chitin of the cuttlebone will hold water in a similar way and that this water will not be easily lost to the circulation.

DISCUSSION

It is a commonplace to say that man is virtually confined to one plane whilst the animals of the sea have a three-dimensional freedom of movement because the sea takes away their weight. Unless an animal is very nearly neutrally buoyant this freedom is, however, very incomplete. A weight in sea water of only a few per cent of the weight in air can only be held up in the yielding medium of the sea by vigorous and continuous effort. If we compare the restless activity of *Loligo* with the effortless hovering of *Sepia* which, as it stalks its prey, can poise in the water to shoot out its tentacles, we may see clearly how the whole behaviour of an animal is determined by its weight in water.

The cuttlebone represents a particularly elegant solution to the buoyancy problem. It is true that it occupies about 9·3% of the animal's volume, compared with the 5% of the gas-filled swimbladder of a fish, but it serves *Sepia* as a skeleton as well as a buoyancy tank. The cuttlebone, which is on the upper side of the animal, seems to be well placed and the anaesthetized cuttlefish lies in its natural swimming position. In man-made machines for travelling in mid water, such as the submarine and the bathyscaphe, changes in buoyancy can be used to give vertical movements in the sea. We have shown that the cuttlefish can change the density of its cuttlebone and we shall see later (Denton & Gilpin-Brown, 1961*a*) that the cuttlefish can use its cuttlebone as a variable buoyancy tank to enable it to become more or less dense than sea water.

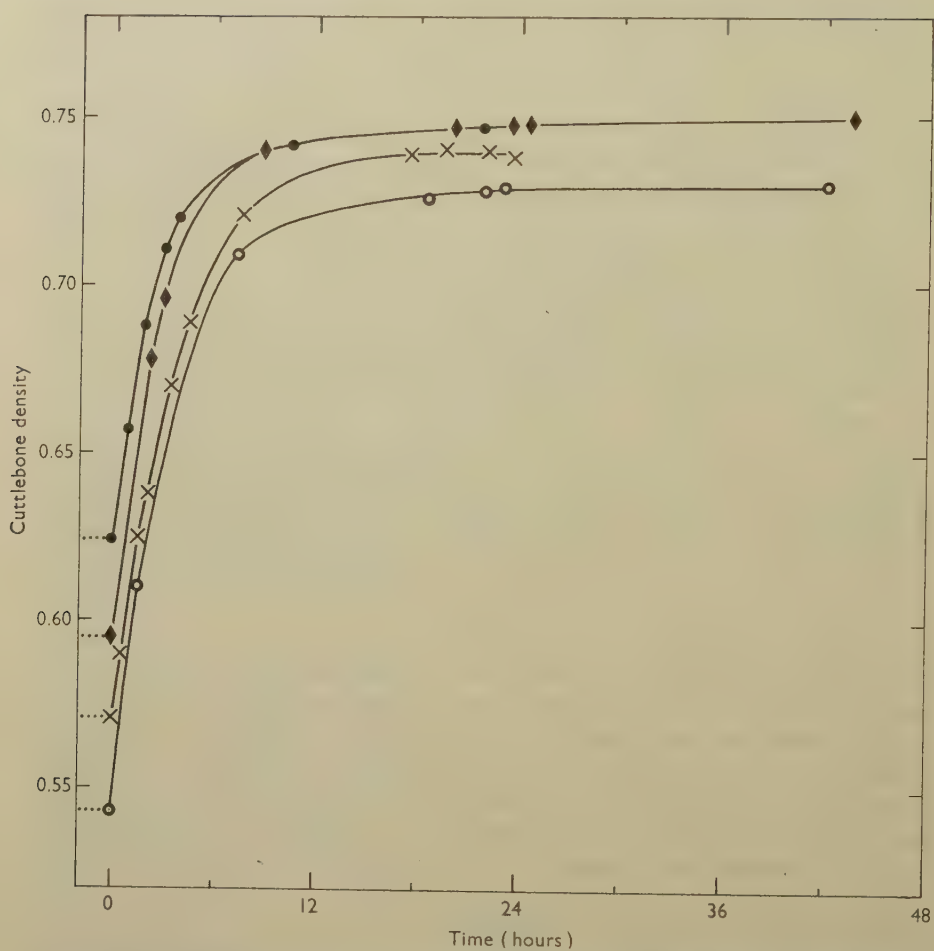
Anatomically the siphuncular wall is the only likely one through which liquid can move in and out of the cuttlebone.[1] It is freely permeable to liquids and the siphuncular membrane covering it has a special drainage system (see Text-figs. 8, 9 and 10). It has also been shown (Denton & Gilpin-Brown, 1961*b*) that the liquid in the cuttlebone is localized at the siphuncular ends of the chambers and that the amount of liquid at these ends increases and decreases as the density of the cuttlebone rises and falls. For these reasons we conclude that in a living cuttlefish the siphuncular wall is the one through which liquid enters and leaves the cuttlebone.

Although this wall is permeable it offers a considerable resistance to the entry of water. We may compare the rates of entry of liquid in Text-figs. 1 and 11. In the cuttlebones of Text-fig. 1 water entered through a hole of about 1 mm^2 made in the bone and the density of the bone rose about half way from its initial to its final value in about 10 sec. In Text-fig. 11, in which water entered through the whole intact siphuncular wall the corresponding time was about 2 hr. In life the siphuncular wall of the bone is covered by the siphuncular membrane and this is fairly impermeable to water. Text-fig. 12 shows experiments on cuttlebones in which the siphuncular surface was partially covered with a siphuncular membrane. It can be seen that the rate at which water is taken up was increased greatly when the remaining membrane was wiped off. Mr A. C. G. Best, of the Plymouth laboratory, has very kindly stained the membrane with a number of fat sensitive stains, but none of these showed a well marked fatty layer. There may of course be a very thin layer of some other material, e.g. wax, which, although effective as a barrier to water, cannot be easily stained. In life the blood system penetrates into the membrane and liquid from the circulation can be brought through the membrane and very close to the surface of the bone, and so allow exchanges of liquid to take place between the blood and the liquid inside the cuttlebone.

In Text-fig. 9 the siphuncular membrane of *Sepia* may be compared with the siphuncular membrane of *Nautilus*, the only living representative of the very numerous fossil Nautiloidea (Willey, 1902). Although the gross anatomy of *Nautilus* is very different from that of *Sepia* (in *Nautilus* the siphuncle lies within a tube passing through the chambers of the shell) their siphuncular membranes are very much alike. We find the same unusual epithelial structure with numerous ampullae close to the bone connecting by small ducts to an anastomosing system of vessels below the membrane which in turn communicate with the siphuncular vein.[2] This great similarity suggests that

[1] Dr H. Mutvei (personal communication) tells us that this wall, unlike the others, contains no calcareous material. It has the same mineralogical constitution as the fragile parts of the siphuncular tube of *Nautilus*.

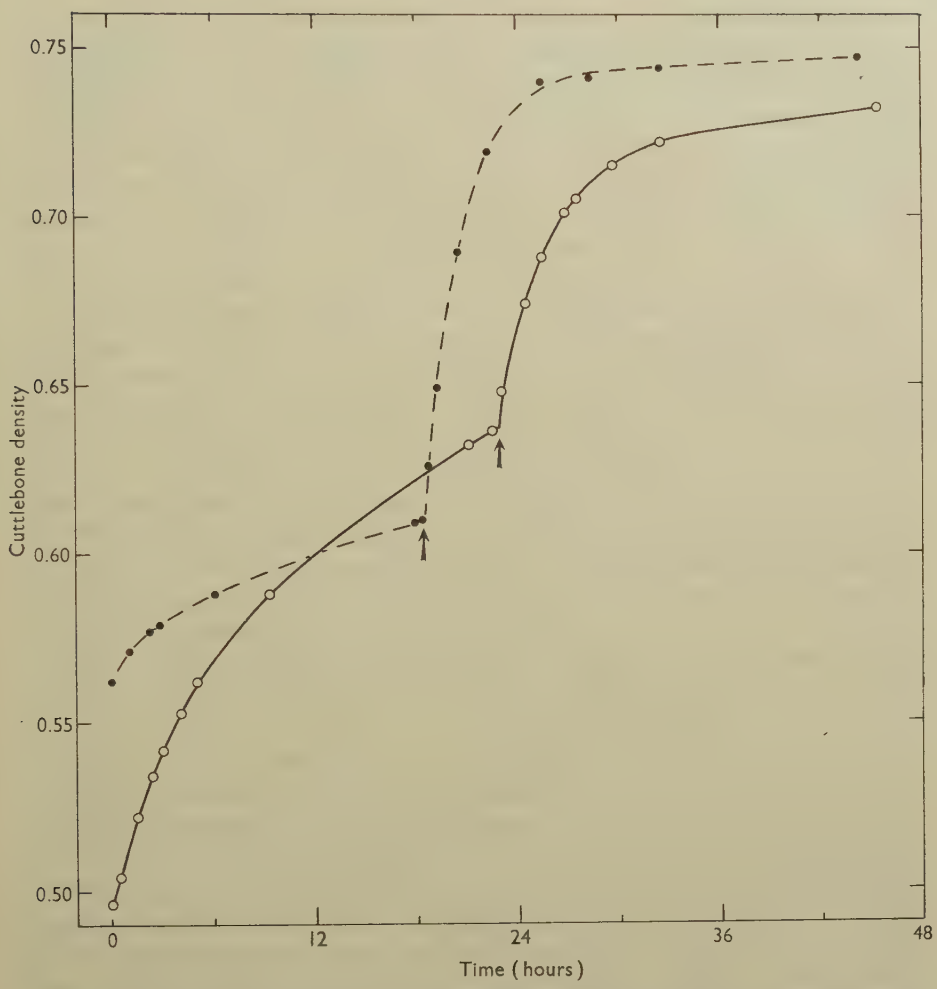
[2] Willey uses a nomenclature different from that adopted in this paper. He describes the ampullae as epithelial spaces and the spaces below the membrane as a trabecular meshwork.



Text-fig. 11. Changes in density of cuttlebones of various initial densities after removal of their siphuncular membranes and soaking in sea water. It will be noted that the ordinate indicates much longer time than that of Text-fig. 1.

the mechanism for moving liquid described here and in the following communications for *Sepia* is also used by *Nautilus*, and hence probably it was also used by the fossil cephalopods.

In the experiments on dissected bones the pressure difference which pushed water into the cuttlebone was that between atmospheric pressure and the pressure of gas within the bone, that is a pressure difference of about 0·2 atm. In life at the bottom of the sea this pressure difference may be one of several atmospheres so that even if the circulation to the cuttlebone were occluded, the membrane (about 1 mm thick) separating the liquid of the visceral cavities



Text-fig. 12. Changes in density of cuttlebones freshly dissected and placed under sea water. The siphuncular membranes of the two cuttlebones were initially only partially intact. On rubbing the residual membranes off at the times indicated by arrows the rate of uptake of water increased sharply.

from the cuttlebone would have to be very impermeable to keep liquid out of the cuttlebone. In this respect the cuttlefish differs from *Nautilus* and *Spirula* where the occlusion of the siphuncular circulation would give a long length over which diffusion would have to take place. If the tissues in the siphuncle could sustain both dehydration and anoxia this diffusion barrier could hold water out of the chambers for a long time. The relations between the cuttlefish and *Nautilus* and *Spirula* are discussed by Denton (1961).

Gas in the cuttlebone

Paul Bert (1867) collected the gas obtained by grinding the cuttlebone under water and showed that it was principally nitrogen with a small percentage of oxygen. Since the gas pressure in the cuttlebone is always less than the external hydrostatic pressure, the gas spaces must be made and maintained by some mechanism other than by the evolution of gas. Thus, although the cuttlefish lives down to depths where the external pressures are appreciable, the cuttlebone does not resemble the swimbladder but is closer to the system used in very shallow water by *Corethra* larvae (Krogh, 1911).

The mechanism used in the cuttlebone is probably an osmotic one (Denton, Gilpin-Brown & Howarth, 1961), and the gas probably simply diffuses into the spaces after they are formed. The *average* pressure of gas varies around 0·8 atm and if this gas is, as Paul Bert found, almost pure nitrogen, this would suggest that the cuttlebone gas is in equilibrium with the tissues around it with respect to nitrogen. Nitrogen in the sea is everywhere in equilibrium with the atmosphere above the sea, where its partial pressure is about 0·8 atm. On this hypothesis we might suppose that the oxygen tension within the cuttlebone is low because it is used up by some metabolic activity. The truth is certainly more complicated than this simple theory would suppose for the pressure of gas varies from chamber to chamber within the bone and is much lower in the newer chambers than the older ones (Denton & Gilpin-Brown, 1961*b*).

Liquid in the cuttlebone

Since the chambers of the cuttlebone are probably formed at a rate of several a month, the process of 'pumping out' a new chamber must be a fairly rapid one. In glass apparatus it is usually difficult to form the first small bubbles which act as foci in the formation of larger bubbles. This is because there are strong forces of cohesion between glass and water molecules, and for this reason glass is 'wettable'. We have seen that the chitin of which the cuttlebone is partly formed is not very wettable and that bubbles form very readily on it. There will not therefore be any special difficulty in forming the first small bubble in a newly completed chamber particularly since large forces, i.e. greater than several atmospheres, must be available to form gas spaces in the cuttlebone when the cuttlefish is at the bottom of the sea.

As we have seen some of the liquid which the cuttlebone contains can be extracted by placing the bone under reduced pressure, the gas inside the cuttlebone then expands and pushes liquid through the siphuncular surface of the bone. This fraction of cuttlebone liquid must be readily available for extraction in the intact animal. Since the average density of cuttlebones in freshly caught animals is 0·62 and densities as low as 0·5 have been found, the cuttlefish can extract more than half the liquid which its cuttlebone

usually contains. The extraction of liquid must be an active process since the pressure gradient is against the loss of liquid.

In brief, just as the submarine commander varies the buoyancy of his craft by increasing or decreasing the water content of its buoyancy tanks, so the cuttlefish varies its density by increasing or decreasing the mass of liquid which the cuttlebone contains. To become less dense the cuttlefish extracts liquid from its cuttlebone thus increasing its gas space; to become denser the cuttlefish lets (or pumps) liquid into the cuttlebone, thus decreasing its gas space.

We wish to thank Mr A. C. G. Best for kindly making the histological preparations, Captain C. A. Hoodless and the crew of R.V. 'Sarsia' for their help, and Mr R. G. Maddock for excellent technical assistance.

SUMMARY

The excess weight in sea water of the living tissues of *Sepia officinalis* (L.) is approximately balanced by the cuttlebone, which accounts for about 9·3% of the animal's volume. The density of cuttlebone varies around 0·6. The cuttlefish without its cuttlebone would be about 4% denser than sea water.

The chambers of the cuttlebone are independent of one another but liquids and gases are free to move within any one chamber.

Animals caught and studied fresh aboard ship exhibited a much less wide range of cuttlebone densities than those kept in an aquarium.

Specimens kept in aquaria vary greatly in buoyancy. These variations result from changes in density of the cuttlebone.

Cuttlebones differ not in the weight of dry matter per unit volume, which is always close to 38%, but in the amount of liquid they contain. A cuttlebone of density 0·7 contains about 30% liquid whereas a cuttlebone of density 0·5 contains about 10% liquid. The remainder of the cuttlebone contains gas, but this gas is at less than atmospheric pressure. The pressure of gas varies around 0·8 atmosphere. Within the duration of the experiments described here, the mass of gas per unit volume of bone remained almost constant whatever the bone's density. The pressure of gas is lower the less dense the cuttlebone. There can be no question of an evolution of gas expelling liquid from a bone when it becomes lighter. The constancy of the mass of gas within the cuttlebone is explained in terms of the slowness of diffusion of gases into the bone.

Liquid is added and removed from the cuttlebone through its siphuncular wall. The reduction of external pressure below atmospheric which is required to extract liquid from the cuttlebone is approximately equal to the difference between atmospheric pressure and the gas pressure inside the bone. Surface

forces do not therefore play an important role in the mechanism of the cuttlebone. The yellowish coloured siphuncular membrane covering the siphuncular wall of the cuttlebone acts as a barrier to the penetration of liquid. Serial histological sections show that it contains numerous ampullae close to the bone, draining into the veins. A very similar system of vessels has been found in the epithelium which lines the siphuncle of *Nautilus*.

The cuttlebone of *Sepia elegans* has physical characteristics similar to those of *S. officinalis*. In *Loligo forbesi* the pen has a volume of only 0·5% of that of the animal and its density 1·2 is greater than that of sea water. The whole animal, including its pen, is approximately 4% denser than sea water.

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THE EFFECT OF LIGHT ON THE BUOYANCY OF THE CUTTLEFISH

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(Plate I and Text-figs. 1 and 2)

By moving liquid in or out of the rigid cuttlebone and so changing the volume of the gas spaces which it contains, the cuttlefish, *Sepia officinalis* (L.), can make itself more or less dense than sea water (Denton & Gilpin-Brown, 1961). The following experiments give an example of the use of this mechanism during the life of the animal.

The behaviour of well fed cuttlefish is greatly affected by light. When the light is bright they usually bury themselves in the gravel at the bottom of their tanks, whilst after twilight they come out of the gravel and swim around until dawn. Here we show that striking changes in buoyancy accompany changes in light intensity. The experiments were of two kinds; the first was designed to study the pattern of behaviour of cuttlefish in a normal day and night light cycle, the second to find out whether changes in behaviour with light intensity are associated with changes in buoyancy.

It is very difficult to provide adequate living room in the Plymouth Laboratory's experimental tanks for large adult *Sepia*. Most of the experiments described here were made on small cuttlefish, of less than 300 g, caught in Plymouth Sound. Some confirmatory experiments were made on large *Sepia* kept in two large cages suspended in the Laboratory's sea-water reservoir.

BEHAVIOUR EXPERIMENT

Methods

Thirteen cuttlefish, 5–10 cm long, were placed under sea-water circulation in a tank 115 × 65 × 35 cm in September 1959. Shell gravel and stones were spread over the bottom of this tank to a depth of about 5 cm, and a black cloth was placed over the only glass side so that natural light only came from overhead and the animals were little disturbed by passers-by. These cuttlefish were fed regularly on small prawns. They remained healthy, and grew in size, until this experiment was made some 7 weeks later.

A camera was set up so that all the tank was included within the 35 mm frame, and electronic-flash photographs were made at intervals during day and night. The flash was sufficiently powerful to overcome daylight during

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the day and of short enough duration to give little if any disturbance to the cuttlefish at night. Photographs such as those shown in Pl. I, were obtained, and these show how many cuttlefish were buried at different times of the day and night.

The illumination was natural diffused daylight, reflected into the top of the tank from the white walls of the laboratory. The fraction of daylight reaching the floor of the tank was found by comparing (with an Ilford SE I photometer) the luminance of a flashed white opal diffusing surface placed on the bottom of the tank with that for the same surface placed in shallow water under an open sky. It was found that the illumination on the floor of the tank was about $\frac{1}{100}$th that outside the building. This is the fraction of daylight in the waveband 450–550 mμ (i.e. that to which *Sepia* is probably most sensitive) penetrating to about 40 m in the sea off Plymouth (Poole & Atkins, 1937; Jenkin, 1938). The cycles of intensity were therefore approximately those to which the cuttlefish would be exposed in their natural habitat. The variations in light intensity during the course of the experiment were measured (with the SE I photometer) on the white wall immediately above the tank so that the cuttlefish were disturbed as little as possible. One of the curves in Text-fig. 1 shows the variations of intensity with time, the light being approximately $\frac{1}{100}$th that of the light outside the building.

Results

In Text-fig. 1 the number of cuttlefish *not* buried and the intensity of light have both been plotted against time. It is clear that at dusk, as the light intensity falls, cuttlefish come out of the gravelly bottom and swim about. This swimming phase lasts throughout the night until dawn when the cuttlefish again bury themselves in the gravel. Although the graph only indicates the numbers above the bottom and not their depth, occasional observations made during the night with a very weak red light always showed some cuttlefish swimming near the surface or in mid-water.

BUOYANCY EXPERIMENT

Methods

For this experiment two cuttlefish of about 330 and 260 g were placed in a tank (85 × 40 × 55 cm) with gravel on the bottom in a dark room.

The weight of a cuttlefish in sea water was found by weighing it (on a torsion balance) under sea water in a watertight polystyrene box filled with sea water. It is quite easy to shepherd an animal into such a box and to make

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Explanation of Plate I

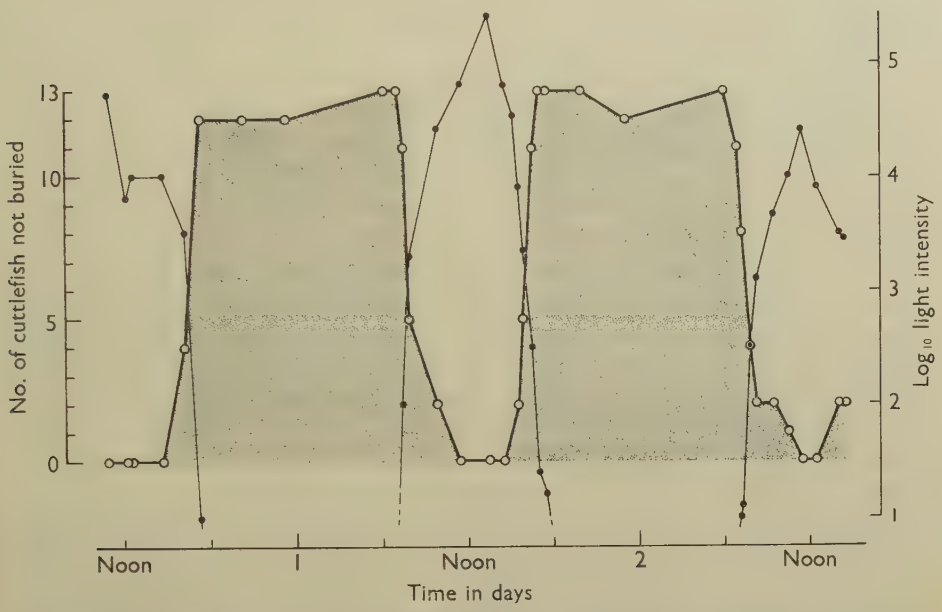
A tank containing thirteen cuttlefish photographed by flash photography. The upper photograph was taken in the night and all thirteen cuttlefish are unburied. The lower photograph was taken in the daytime and all thirteen cuttlefish are buried.

Night

Day

(*Facing p.* 344)

sure that no bubbles of gas or bits of gravel are present. If an animal weighs −3 g in sea water then the weight of the box + animal + sea water will be 3 g less than the weight of the box filled with sea water only. A cuttlefish suffers no ill effects from repeated weighings of this kind, and a record of its changes in buoyancy with time can be made.



Text-fig. 1. *Sepia officinalis*. Diurnal changes in the behaviour of the animals. ○, Number of animals not buried (total number of animals 13). ●, Changes in natural light intensity on a logarithmic scale. The light corresponds in intensity roughly to that found in the sea off Plymouth at 40 m but it differs somewhat in its spectral composition.

At the end of an experiment the animal was killed, the mantle cavity cut open and examined for air bubbles, and the animal weighed directly in sea water. The figure obtained by this direct weighing always agreed with that calculated from the measurement made in the box just before death. Later the animal was dissected to see whether there were any stones in the gut.

An automatic time switch was set to put the light on at 10.00 h and off at 22.00 h every day. The light source was a 100 W tungsten pearl lamp suspended 65 cm above the floor of the tank.

Results

A pilot experiment on one animal is shown in the lower right-hand corner of Text-fig. 2. The animal had been kept overnight in the dark and measurements were started as soon as the light was turned on. At this time it was

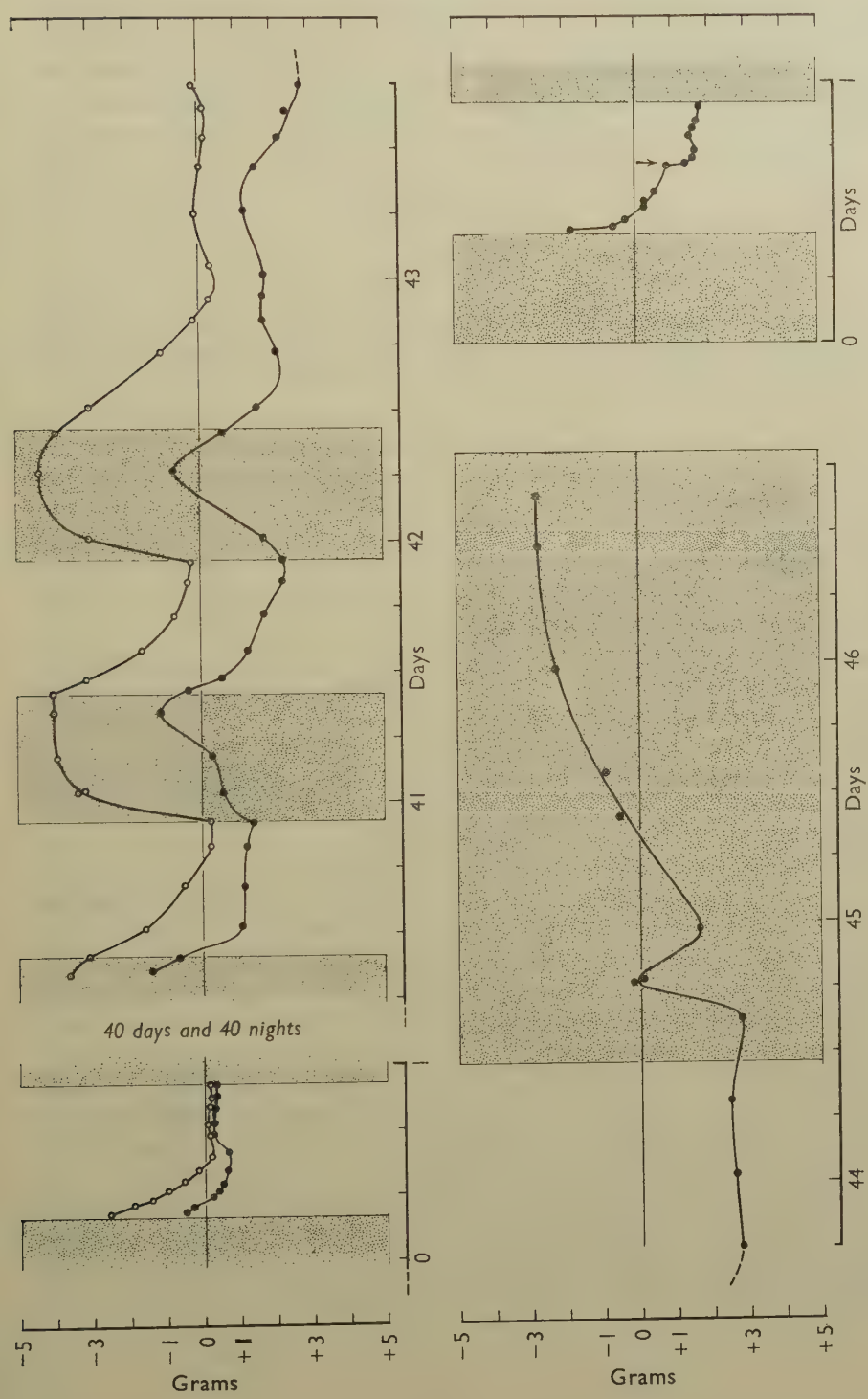
less dense than sea water, but its density increased fairly rapidly so that at the end of the day it was denser than sea water. At the time indicated by the arrow the cuttlefish fed on prawns. Prawns are denser than sea water and produced an immediate increase in the density of the cuttlefish. Apart from the temporarily added weight of the prawns, the cuttlefish continued to decrease its buoyancy at a smooth rate throughout the day. The fact that the animal took and ate its food normally shows that it was little disturbed by the weighings.

The rest of the graph in Text-fig. 2 (top and lower left) shows an experiment made on two individuals. On the first day both animals showed marked increases in density during the period of light. These animals were now left for 40 days under the same alternating periods of dark and light, the weighings were then resumed and the buoyancy changes of the cuttlefish followed through both dark and light periods. A weak red light was used for weighings during the dark periods. A clear-cut pattern of buoyancy change is shown; the animals increasing their buoyancy during the dark periods and decreasing their buoyancy during the light periods. When the dark period was omitted and the light kept on there was only a very slight decrease in density at the time when the dark period would normally have begun. When animals are left continuously in the dark they become, and remain, very buoyant. Two other cuttlefish were studied in this way and they both showed changes in buoyancy with light similar to those shown in the figures.

At the end of the experiments the animals were killed at different states of buoyancy, their cuttlebones dissected out, and their densities found. Finally the cuttlebones were punctured under sea water and the uptake of sea water measured. A dense cuttlefish, killed after a period in the light, had a cuttlebone of high density 0·65 which rose to 0·75 on puncturing under sea water. A second cuttlefish died when near neutral buoyancy and had a cuttlebone of density 0·62, rising on puncturing to 0·74. Two cuttlefish brought to low density by a period in the dark, had cuttlebones with the low densities of 0·54 and 0·55, which rose to 0·75 and 0·78 respectively on puncturing under sea water. As found earlier (Denton & Gilpin-Brown, 1961) no matter what the initial density of the cuttlebone the final density on puncturing is almost constant. This shows that the mass of gas within the cuttlebone remains almost constant when the cuttlebone density is changed.

Legend to Text-fig. 2

Text-fig. 2. The ordinate shows the weight of a cuttlefish in sea water (a negative weight means that the animal is less dense than sea water). The dark areas indicate times of darkness. *Lower right-hand corner.* Change in weight in sea water of a cuttlefish which had been kept for 12 h in the dark and was then subjected to 12 h light. At the time marked with an arrow the cuttlefish ate two prawns. *Top, continued lower left.* Change in weight of two cuttlefish. The upper curve is for an animal varying in weight around 330 g, the lower curve for an animal varying around 260 g.



Text-fig. 2. For legend see opposite page.

During these experiments high and low densities of cuttlebone were sometimes maintained for several days before killing the cuttlefish. The earlier conclusion (based on puncturing experiments, Denton & Gilpin-Brown, 1961) that the rate of exchange of gas between the tissues of the cuttlefish and the gas of the cuttlebone is extremely slow is much strengthened by this observation.

No gas bubbles were found either in the mantle cavities or in the tissues of these animals, and no gravel or other heavy material could be found in their alimentary canals.

DISCUSSION

The behaviour experiment (Text-fig. 1) establishes that young cuttlefish show marked changes in behaviour following the natural light cycle of day and night. The cuttlefish, which were kept under as near natural conditions as possible, grew and remained healthy for many months. One specimen, captured in September 1959, lived for an experiment eventually performed in May 1960. There is little reason to suppose that the behaviour which they showed in the laboratory experiments was different from that in the sea. Large animals kept singly or in pairs within large cages (4 ft. cube), with thick gravel floors and suspended in the Laboratory's sea water reservoir, showed a similar pattern of behaviour. Direct observation of these animals showed that, provided they were well fed (one or two crabs per day), they were buoyant and swimming in mid-water during the night and were buried in the gravel during the day. There are, however, occasions when they do not bury themselves during the day. For instance, if large cuttlefish are hungry they remain swimming in mid-water until they are fed when they immediately return to the bottom and bury with their food. Again, if two males are placed together they sometimes circle each other in mid-water for many days irrespective of the lighting conditions.

The buoyancy experiments show that with changes in light there are marked changes in the buoyancy of the cuttlefish. These changes, for the four animals measured, amounted to 1·0, 1·5, 1·5 and 2·0% of the animal's weight in air. These could scarcely come from changes in protein content for they would demand fluctuations of total protein of between 25% and 50% in animals weighing about $\frac{1}{3}$ kilogram and eating about two prawns per day each. Since they did not swallow stones, the large changes in density could only come from changes in the volumes of gas spaces within the animals. There was no trace of gas in the tissues of the animals and, when the experiments were stopped, the densities of their cuttlebones were close to those predicted on the hypothesis that the cuttlebone was the site of the density changes.

It still remains to show that all the changes in cuttlefish densities with change in light can reasonably be explained in terms of the range of cuttlebone densities already recorded. Knowing the buoyancy of a cuttlefish and the density of its bone at death, we can calculate the density which the bone would

have had at other times during the experiment. The calculated values for cuttlebone densities when these four cuttlefish were at minimum, maximum, and neutral buoyancy are given in Table 1. There they are compared with the maximum and minimum values found by Denton & Gilpin-Brown (1961) for a number of adult cuttlefish kept in the laboratory. The range given on these four animals, 0·77–0·48, is somewhat greater than the range 0·71–0·48 found earlier. This is only to be expected because this experiment, with its long periods of light and dark, was designed to send the cuttlefish into abnormally high and low states of buoyancy. When kept in the dark for long periods cuttlefish become so buoyant that they are incapable of staying on the bottom and can only remain in mid-water with difficulty. Typically such a cuttlefish can swim head down to the bottom of the tank, but when it tries to level out in preparation for burying it at once starts to float upwards

TABLE 1

Cuttlefish	Calculated density of the cuttlebone when the cuttlefish was at: Minimum buoyancy	Neutral buoyancy	Maximum buoyancy
A	0·65	0·61	0·53
B	0·64	0·63	0·48
C	0·77	0·65	0·54
D	0·69	0·67	0·54
Range of densities already recorded (Denton & Gilpin-Brown, 1961)	0·71	—	0·48

again. In the experiment of Text-fig. 2 the cuttlefish were subjected to total darkness for 12 h and the low cuttlefish densities obtained during the dark periods are certainly lower than normal. In the sea since the optimum buoyancy for swimming is probably near neutral (i.e. zero weight in water) only a small change in buoyancy need occur between day and night.

The maximum rates of change in weight in sea water for one animal (Table 1, B; Text-fig. 2, upper curve) were 1·5 and 1·4 g/h when getting lighter and 0·7 and 0·66 g/h when getting heavier. This cuttlefish weighed 336 g in air and its cuttlebone had a volume of 30·4 ml., so that this animal could lose weight in sea water at a rate equal to 5 g/h/100 ml. of cuttlebone. Similarly, it gained weight in sea water at a rate equal to 2·3 g/h/100 ml. of cuttlebone. Such a rate of change would enable a cuttlefish to go from being slightly heavier (say 0·2 %) than sea water when buried in the bottom, to a neutral state for swimming in less than half an hour. A cuttlebone from which the siphuncular membrane has been removed and which is soaked in sea water gains weight at a maximum rate of about 5 g/h for 100 ml. of cuttlebone (Denton & Gilpin-Brown, 1961). This rate is more than twice that found in the intact animal, and it is therefore unnecessary to assume that

any active process is used in pumping liquid into the cuttlebone when a cuttlefish makes itself denser. The cuttlefish must, of course, do active work when it extracts liquid from the cuttlebone to become less dense.

These experiments show then that the cuttlefish is able to change its density at quite rapid rates and almost certainly does so to help it lie at the bottom during the day and to keep it off the bottom during the night.

We wish to thank Mr A. C. Briggs for collecting young cuttlefish in exceptionally good condition and Mr F. J. Warren for the loan of his camera.

SUMMARY

The behaviour of well fed cuttlefish is strikingly affected by light. When the light is bright they usually bury themselves in the gravel at the bottom of their tanks, whilst after twilight they come out of the gravel and swim around until dawn.

Cuttlefish kept in complete darkness for one to two days usually become so buoyant that they are incapable of staying on the bottom and can only remain in mid-water with difficulty.

Animals kept in artificial light and dark showed changes in density which could amount to 2 %. These changes come from changes in the volume of the gas space within the cuttlebone.

The density of the cuttlefish is largely governed by light intensity and there is little indication of an inherent diurnal rhythm of density change.

The rate of uptake of liquid when the cuttlefish becomes denser is such that it is unnecessary to assume that any active process is used to pump liquid into the cuttlebone. The cuttlefish must, however, extract liquid actively from the cuttlebone to become less dense.

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THE OSMOTIC MECHANISM OF THE CUTTLEBONE

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(Text-figs. 1–6)

The cuttlefish can vary its density by moving liquid into or out of its cuttlebone and so varying the volume of the gas space which the cuttlebone contains. The gas pressure is always less than atmospheric and falls when liquid is pumped from the bone and there can be no question of this gas pressure balancing the external hydrostatic pressure of the sea (Denton & Gilpin-Brown, 1961*a*). Since the cuttlebone is not an impermeable structure some other mechanism must be used for the pumping of liquid. This communication shows that osmotic forces are probably used to control the volume of the gas space within the cuttlebone.

The great defect of the swimbladder of a fish as a buoyancy mechanism is that, since the swimbladder wall is not rigid, its volume changes greatly with depth (Moreau, 1876). The fish is in a state of unstable equilibrium for if it rises in the sea the swimbladder will expand and tend to push it still higher whilst if it goes deeper in the sea the swimbladder will be compressed and the fish will tend to sink more and more quickly. Here we show that within the cuttlebone the volume of the gas space remains approximately constant with change in external pressure and that it will therefore act as a buoyancy mechanism which is almost independent of depth.

MATERIAL AND METHODS

Material

Experiments on freshly caught *Sepia officinalis* (L.) were made aboard R.V. 'Sarsia' in the spring of 1959 and the spring of 1960. At this time of year the animals are very rarely found in the English Channel off Plymouth in depths of less than 35 fathoms and we can therefore be fairly sure that the animals used, which were taken at about 40 fathoms, had not recently migrated there from shallower water. As soon as possible after the trawl came inboard the animals were placed in sea-water circulation in the ship's tanks. Animals not used at sea were kept in two large cages in the large sea-water reservoir of the Plymouth Laboratory.

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Extraction of liquids

Blood was taken from the cephalic aorta.

To extract liquid from the cuttlebone the following method was used. A cuttlebone was dissected out quickly and the yellowish membrane covering the siphuncular surface was gently removed. The bone was then placed, ventral side upwards, in a bath of liquid paraffin inside a vacuum desiccator. The pressure within the desiccator was gradually reduced and the liquid which came through the siphuncular surface trickled down to collect in the cup formed by the curved posterior dorsal part of the bone (see Fig. 1). When sufficient liquid had collected, atmospheric pressure was restored and the liquid removed by Pasteur pipette. Successive samples of liquid from inside the cuttlebone were obtained by progressively reducing the external pressure on the bone.

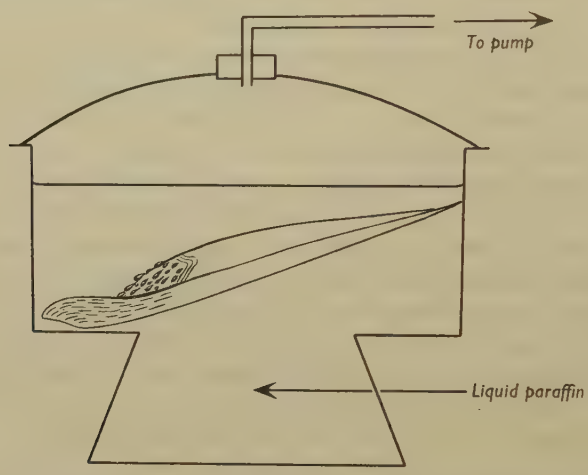

Fig. 1. Apparatus used to extract liquid from within the cuttlebone. Drops of liquid can be seen coming from the siphuncular surface of the bone.

The samples of blood, sea water, and cuttlebone liquid which were to be used for later analysis were stored under liquid paraffin, in sealed tubes which were placed in sealed Kilner jars in a deep freeze. Kilner jars were used so that the samples could be later brought back to room temperature without condensation of water on the tubes containing samples.

Determinations of osmotic pressure and chemical analyses

Osmotic pressures were determined by two methods. In 1959 the freezing-point depression was determined by a method similar to that used by Freeman & Rigler (1957). In 1960 the thermoelectric method of Hill and Baldes was used: the chamber used was similar to that described by Krogh (1939) but,

at Dr Baldes's suggestion, this was placed not in a water bath but in air in a vacuum flask. Both methods gave results which were reproducible to 2% of the tonicity of sea water.

Analyses of chloride were made using the micro-diffusion method of Conway (1940); sodium and potassium were measured with an EEL flame photometer. In all analyses the unknown solutions were compared with sea water. The sea water used as a standard was usually that taken at sea close to the place where the animals were caught. Since, the sea water in the reservoirs of the Plymouth Laboratory usually differs a little from that at sea, this was used as a standard when studying animals which had been kept for some time in the reservoirs.

The effect of pressure on the buoyancy of the cuttlefish

A small cuttlefish was placed in a very strong metal cylinder of volume 1250 ml. designed to contain an instrument used for work in the deep sea. The cylinder was closed at one end by a thick Perspex lid and connected by a tube going through this lid to a pump and a manometer. The cylinder and

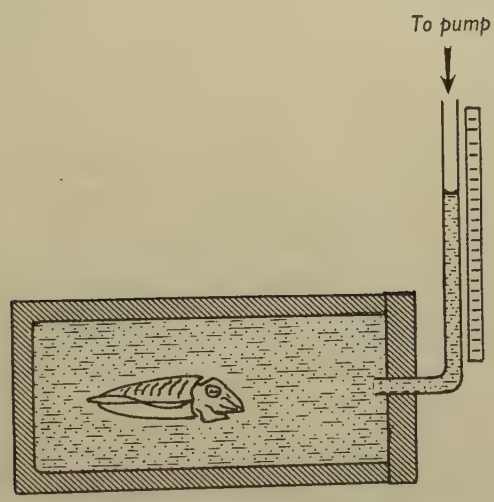

Fig. 2. Apparatus used to study the effect of pressure on the volume of the gas spaces within a cuttlefish and a pollack.

part of the tube were filled with sea water (Fig. 2) and changes in the volume of the contents of the cylinder with pressure could be measured in terms of the height of the sea water in the tube. Similar measurements were made on a young pollack.

RESULTS

Osmotic pressure

Both in 1959 and 1960 the animals were trawled in about 73 m. The results obtained using the depression of freezing-point in 1959 and the vapour pressure method of Hill and Baldes agreed very well; they are shown

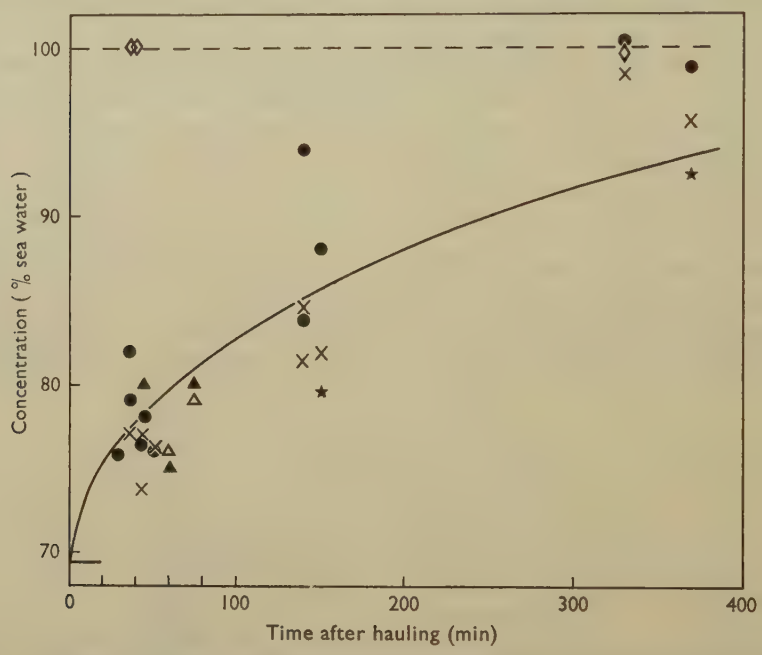

Fig. 3. Cuttlefish have been taken from about 73 m. The ordinate is osmotic concentration as a percentage of sea water. The abscissa is time in minutes between the start of the haul and the dissection of the cuttlebone. ▲, and △ are results obtained by measurement of freezing-point in 1959. They are respectively, for the first and second samples of cuttlebone liquid extracted. ●, ×, ★ are results obtained by the vapour pressure method in 1960. They are respectively for first, second, and third samples of cuttlebone liquid extracted. ◇ is for cuttlefish blood. The thick horizontal line perpendicular to the ordinate at about 70 % sea water gives the value predicted for the liquid when the cuttlefish was at the bottom of the sea. The graph is a curve calculated (see p. 360) on the assumption that the rate of equilibration of cuttlebone liquid on change of depth is determined by diffusion. No arbitrary constants are needed in this calculation.

on Figs. 3 and 4. It can be seen that in the first 6 h after capture the osmotic concentration[1] of the cuttlebone liquid changes from about 75% sea water to close to 100% sea water. When successive samples were taken from a cuttlebone the later ones i.e. those from deeper inside the bone, had the lower osmotic concentrations (Fig. 3).

[1] The concentration of a liquid extracted from a cuttlebone is here given in terms of the dilution of sea water which is osmotically equivalent to it; i.e. a dilution which at the ambient temperature has the same vapour pressure, or which has the same freezing-point. This concentration will be referred to as the osmotic concentration.

The blood always had an osmotic concentration very close to that of sea water. After keeping two cuttlefish for several weeks in shallow water in the laboratory both blood and cuttlebone liquid were within 2% of the osmotic concentration of sea water.

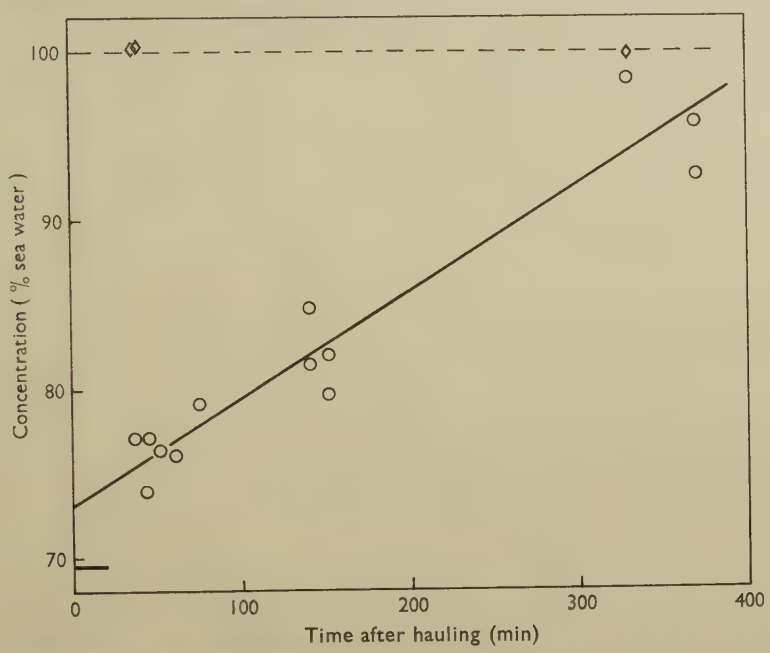

Fig. 4. The data in Fig. 3 relating to the 2nd and 3rd (i.e. the deeper) samples have been replotted. The *minimum* predicted osmotic concentration before capture is shown by a thick black line just below the 70% value on the ordinate. A straight-line extrapolation has been made back to the time when hauling began, although this is probably unfavourable to the osmotic hypothesis. It can be seen that the osmotic concentration of the cuttlebone liquid before capture must have been close to that predicted.

Chemical analyses

Fig. 5 shows that the differences in osmotic concentration between cuttlebone liquid and sea water are mainly due to reductions in the two principal ions, sodium and chloride. The concentration of sulphate which is very low in cuttlefish blood (Robertson, 1949, 1953) is also very low in cuttlebone liquid. This was shown by a qualitative test in which the precipitates given by adding acidified barium chloride solution to cuttlebone liquid and sea water were compared.

Effect of pressure

Before the experiment the cuttlefish which weighed 203 g in air was weighed in a box filled with sea water under sea water (Denton & Gilpin-

Brown, 1961*b*). The weight in sea water of the cuttlefish was found to be +0·50 g. After subjecting the animal to the cycle of positive pressures described below and then returning to atmospheric pressure its weight in sea water was found to be +0·57 g and thus little changed. The cuttlefish was then killed and the cuttlebone was dissected out of the animal. Using the methods described earlier (Denton & Gilpin-Brown, 1961*a*), its volume was found to be 17·9 ml. and its density 0·70. The volume of the gas space

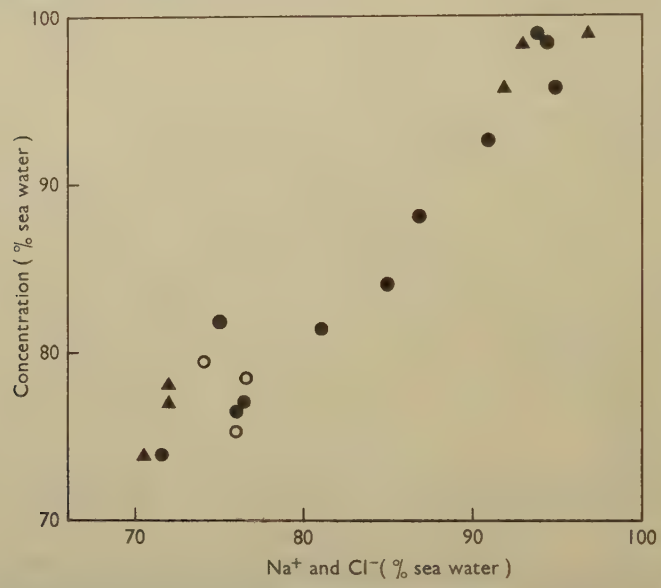

Fig. 5. Analyses made on cuttlebone liquids of various osmotic concentrations. The abscissa shows sodium and chloride concentrations as a percentage of sea water. ○, Sodium (1959 samples); ●, sodium (1960 samples); ▲, chloride (1960 samples). The ordinate is the osmotic concentration as a percentage dilution of sea water.

was estimated as being 9·3 ml. In the living animal pressure was applied in steps of 1 atm to a pressure of 5 atm above atmospheric. The animal was left at this pressure for 15 min and then decompressed quickly. The animal was in the pressure chamber for about 40 min. The changes in volume with pressure were almost the same for the cylinder containing the cuttlefish and the cylinder containing only sea water. The maximum change attributable to the cuttlefish was less than 0·13% of the estimated volume of the gas space in the cuttlebone.

For comparison the volume changes of a young pollack weighing 24·5 g were measured for the same cycle of pressures in the same apparatus. Very appreciable changes in volume were now found. We may safely assume that the very compressible element in a pollack is the gas-filled swimbladder which does not have a rigid wall like that of the cuttlebone. On plotting volume

change against 1/pressure a straight line is given and, by extrapolating back to $1/p = 0$ where the volume would be zero, we can estimate the volumes of this gas at other pressures. The volume of gas at one atmospheric pressure corresponded to 4·6% of the volume of the fish. This is close to the value expected for this fish had lived for some time in shallow water in the aquarium before the experiment.

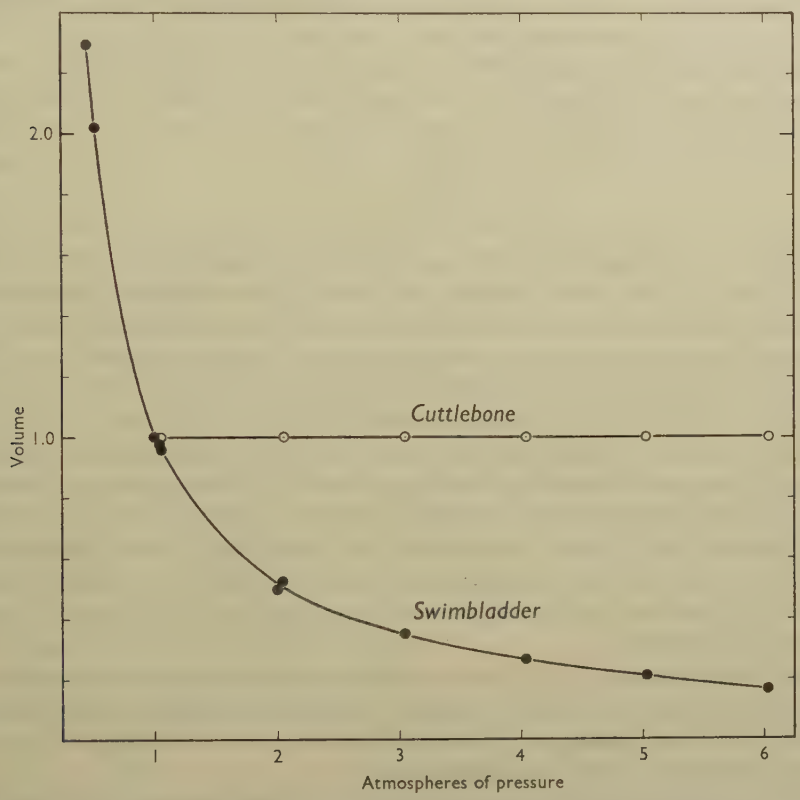

Fig. 6. The change in volume of gas space with pressure: ○, in the cuttlebone; ●, in the swimbladder of a pollack. The volumes of the gas spaces at one atmosphere pressure and room temperature are taken as unity.

Fig. 6 shows both the change in volume of the cuttlebone gas-space (in the living cuttlefish) when the pressure is changed and also the volume change of the gas in the swimbladder of the pollack. The volumes are given with respect to the volumes at 1 atm pressure and room temperature. Since the volume of the gas space of the cuttlebone was about eight times that of the gas in the fish's swimbladder there can be no question that the method was sensitive enough to detect any but the smallest changes in the volume of the gas space in the cuttlebone.

Effects of high pressures on the cuttlebone

Mr R. Dobson of the National Institute of Oceanography kindly tested the effect of pressure on a cuttlebone. This was placed in a closely applied polythene bag, to prevent it merely filling with water, and the pressure increased in steps of 1 atm. The cuttlebone was decompressed and examined between each step. It was found to be unaffected by pressures up to 23 atm, but at 24 atm a loud cracking noise was heard and the cuttlebone was found to have imploded from its curved ventral side. Cuttlefish are usually taken between 30 and 80 fathoms (5–15 atm) and would not therefore be exposed to pressures sufficient to break their cuttlebones.

DISCUSSION

The hypothesis which we are testing is that the hydrostatic pressure of the sea, tending to push water into the gas space of the cuttlebone, is balanced by an osmotic difference between the blood and the cuttlebone liquid. This osmotic difference is probably maintained across the membrane which covers the siphuncular wall of the cuttlebone, there being some mechanism which can pump salts or water to produce and maintain this difference.

There have been relatively few good direct measurements of osmotic pressure, and such measurements are particularly difficult to make on salt solutions which often damage the artificial membranes of copper ferrocyanide used (Morse, 1914). There is therefore some uncertainty as to the osmotic pressure which would, for example, be given between 75% and 100% sea water acting across a membrane permeable to water but impermeable to all the salts of sea water.

Clapeyron's equation allows us to predict the osmotic pressure of a solution from a knowledge of the depression of its freezing-point below that of the pure solvent, the latent heat of fusion of the solvent and the change in volume on freezing (see, for example, Fabry, 1933).

If an osmotic pressure of 22·41 atm could be given between a given solution and water at 0° C, then Clapeyron's equation would predict that the freezing-point of this solution would be −1·85° C (Morse, 1914) [22·41 atm is the pressure exerted by a gram molecule of a perfect gas with a volume of 1 l. at 0° C]. From a knowledge of the osmotic pressure at the freezing-point we may predict the osmotic pressure at another temperature by assuming that it is proportional to the absolute temperature. This assumption is reasonably sound for temperatures close to that of the freezing-point. Morse showed that a solution of cane sugar with a freezing-point depression of 1·829° C gave at 0° C an osmotic pressure (against water) of 22·13 atm (calculated value 21·99 atm), whilst at 15° C the osmotic pressure was 23·31 atm (calculated value 23·20 atm); above 25° C he found that the osmotic pressures

were greater than those predicted. We shall assume: (1) that since the freezing-point depression of the standard sea water used was $-1{\cdot}91^\circ$ C instead of $-1{\cdot}85^\circ$ C (its salinity was 35·25‰) the osmotic pressure between it and pure water would be $(22{\cdot}41 \times 1{\cdot}91)/1{\cdot}85$, i.e. 23·1 atm at 0° C; (2) where we have solutions of two different freezing-points the osmotic pressure which would exist between them is proportional to the difference in freezing-point depressions, for example between solutions of freezing-point depressions 1·91° C and 1·39° C the osmotic pressure difference at 0° C would be $[23{\cdot}1 \times (1{\cdot}91 - 1{\cdot}39)]/1{\cdot}91$ atm; and (3) the osmotic pressure difference between two solutions is proportional to the absolute temperature. At 10° C, the temperature of the sea where the animals were caught, the osmotic pressure difference between sea water and pure water would be 24·0 atm.

In the measurements of vapour pressures we are matching our unknown solutions against diluted sea waters. Now the galvanometer deflection given between pure water and 25% sea water was very nearly the same as that between 75 and 100% sea water; this shows that the vapour pressure differences are approximately the same. We shall assume that the osmotic pressure is proportional only to difference in concentration. This means, for example, that at 10° C the difference in osmotic pressure between 75 and 100% sea water would be $[24{\cdot}0 \times (100 - 75)]/100$ atm. The sea waters were diluted by adding known volumes of sea water to known volumes of distilled water; for example, 50% sea water was obtained by mixing equal volumes of sea water and distilled water. This method of dilution differs from that recommended by Morse who used the weight-normal system. The errors given by neglecting these differences are very small and are unfavourable to the osmotic hypothesis advanced here.

The animals used were caught in 73 m and for them the *maximum* pressure pushing water into the cuttlebone would have been 8·2 atm. Only about 0·8 atm of this pressure would be balanced by the gas pressure inside the cuttlebone leaving, on the present hypothesis, 7·4 atm to be balanced by the osmotic difference between blood and cuttlebone liquid. Since the blood has the same osmotic concentration as sea water this requires that the cuttlebone liquid should have a concentration of 69·2% sea water. Figs. 3 and 4 show that the composition of liquid within the cuttlebone changes very appreciably in the first few hours following the hauling of the trawl. About 40 min. after the start of the haul the samples from deeper inside the cuttlebone had osmotic concentrations of about 75% sea water, whilst 6 h later they were equivalent to about 97% sea water. To estimate the concentration which the liquid would have had at the bottom of the sea we may extrapolate back to the time at which the haul began. It can be seen from Fig. 4 that before capture the concentration of salts in the cuttlebone liquid would have been reasonably close to the value of 69·2% sea water predicted. If the

cuttlefish had been above the bottom of the sea before capture then the expected value would have been higher than 69·2%.

How can we interpret the fact that, in the first hours following capture, the liquids deeper within the cuttlebone differ from those nearer the siphuncular surface? This presumably comes about because diffusion limits the rate at which complete equilibrium can be re-established after a change in depth. Taking conditions similar to those of our experiments, let us suppose that an animal is living at 73 m depth and that the cuttlebone liquid is uniform in composition and of a concentration equivalent to 69·2% sea water. The animal is then brought into very shallow water and after some time re-establishes equilibrium with a concentration of salts in the cuttlebone liquid everywhere equal to 100% sea water. Let us try to calculate the way in which this change takes place. We shall assume, for the sake of simplicity of calculation, that the volume of liquid within the cuttlebone remains constant and that the membrane covering the siphuncular surface immediately re-establishes and then maintains equilibrium between osmotic and hydrostatic pressures, i.e. it keeps the concentration of salts just inside the siphuncular surface equal to that of sea water. Until equilibrium is completely re-established the concentration of salts just inside the siphuncular surface will be higher than that deeper within the cuttlebone and salts will diffuse inwards. The membrane will continuously have to pump more salts to balance those diffusing away and thus ensure that there is no concentration difference across the membrane itself. Now the liquid within the cuttlebone is confined in very narrow channels by the lamellae, and mixing will not readily take place. The exchange of salts between the liquid just inside the siphuncular surface and that deeper within the cuttlebone will be limited by diffusion. This surface has an area of about 16 cm^2 in an average-sized cuttlebone and on the average about 3 ml. of liquid were extracted from a bone. Allowing for the great inclination of the siphuncular surface to the lamellae and for the solid matter of the cuttlebone we arrive at a depth of liquid along the channels of about 0·75 cm. We are only concerned here with the liquid extracted under reduced pressure from the siphuncular surface.

This problem of the change in distribution of salts within the lamellae is roughly similar to a problem in diffusion solved by Hill (1928, pp. 55–69). He considers the diffusion of oxygen into a parallel-sided plane sheet of thickness b, bounded on one side by an impermeable wall and exposed on the other wall at time $t = 0$ to a maintained change in concentration of oxygen. He gives (his Table 3) the average saturation of the sheet with oxygen for various times following this change. By substituting, in his equations, the value for the diffusion constant of the salts in sea water, here taken as equal to the $1{\cdot}39 \times 10^{-5}$ cm^2/sec given for sodium chloride in water (International Critical Tables, cited by Höber, 1945) and 0·75 cm for b, we can find the way in which the average concentration of salts within the

cuttlebone will change with time following a change in depth. This is shown by the curve of Fig. 3.

It can be seen that the times which the calculation predicts for given changes in concentration of salts within the cuttlebone are in fair agreement with those found by experiment. After about 60 min the average concentration of salts would have risen from 69·2 to 80% of sea water, at 250 min to about 90% of sea water.

The explanation given above is certainly too simple, for it seems certain that some net exchanges of water take place in the first few hours following change in depth. We expected that, on coming to the surface and so reducing the hydrostatic pressure, the osmotically stronger blood would draw water from the cuttlebone liquid. In the experiments we were surprised to find that greater volumes of liquid could be extracted from the cuttlebones of cuttlefish taken 2 h after capture than those used in the first hour after capture.

Nevertheless, whatever the actual exchanges of salt and water which take place between cuttlebone and blood on change in depth of the cuttlefish, it seems certain enough that the slowness of diffusion of salts within the cuttlebone liquid will greatly limit the rate of equilibration. This is clearly a very advantageous arrangement for the cuttlefish. If, for example, it swims upwards and stays for 1 or 2 h near the surface of the sea, a small exchange of salt or water across the siphuncular membrane can re-establish the balance across the siphuncular wall between osmotic and hydrostatic pressures. The buoyancy of the cuttlefish need hardly change. The liquid deeper inside the cuttlebone will preserve a concentration close to that suitable for the bottom of the sea, where the cuttlefish probably spends most of its life. When the animal returns to the bottom of the sea very little work will have to be done to re-establish complete equilibrium.

We may give a numerical example; consider a cuttlefish at 70 m (i.e at 8 atm pressure) in which all the liquid inside the cuttlebone is osmotically equivalent to about 70% sea water. The animal now swims to the surface (1 atm pressure). The liquid just inside the siphuncular wall is made osmotically equivalent to 100% sea water and a balance between osmotic and hydrostatic pressures across the siphuncular wall is restored. Owing to the slowness of diffusion, liquid far from the siphuncular wall retains for several hours a concentration approximately equivalent to 70% sea water and will not have to be changed when this animal swims down again to the sea bed.

This buoyancy system would be expected to be relatively insensitive to changes in depth and to compare very favourably with that of the swimbladder of a fish where changes in depth, particularly those near the surface, produce great changes in the volume of the gas space of the swimbladder and hence of the fish's buoyancy. This is what the experiment on pressure showed to be true.

An osmotic difference between cuttlebone liquid and cuttlefish blood can hold water out of the cuttlebone, but it cannot balance the crushing effect of

the pressure of the sea. The cuttlebone is, however, very strong. Its structure, with plates held apart by numerous pillars, is especially well suited to sustain compression and we find that it only breaks under pressures greater than those found in the animal's normal depth range.

We wish to thank Dr T. I. Shaw for very helpful discussions and Mr F. G. C. Ryder for making the apparatus used for vapour pressure determinations.

SUMMARY

Experiments are described which test the hypothesis that the cuttlefish controls the relative volumes of gas space and liquid within its cuttlebone by an osmotic mechanism acting across the siphuncular surface of the bone. When the animal is at the bottom of the sea it would maintain the gas space within the cuttlebone by balancing the hydrostatic pressure of the sea by an osmotic force between the liquid within the cuttlebone and the blood.

In cuttlefish kept for some weeks in shallow water in an aquarium all the liquid taken from the cuttlebone is almost isotonic with the animals' blood.

In animals recently hauled from the bottom of the sea the cuttlebone liquid is markedly hypotonic to sea water and hence to the blood.

These lower osmotic concentrations are given chiefly by reduction in the concentrations of the sodium and chloride ions.

After bringing animals up from about 70 m to the surface of the sea the osmotic concentration of the cuttlebone liquid rises from about 75% of sea water some 40 min after starting to haul the trawl, to about 97% of sea water 6 h later. Extrapolation back to the time at which hauling began gives a concentration of salts close to that predicted by the osmotic hypothesis.

Whilst the cuttlebone liquid is increasing in ionic concentration the liquid deeper in the cuttlebone is hypotonic to that just inside the siphuncular surface. This is explained in terms of the slowness of exchange of salts along the narrow channels between the lamellae of the cuttlebone. On the assumption that diffusion limits this exchange the magnitude of the effect is calculated and shown to be in fair accord with the experimental results.

In the living cuttlefish external pressure, at least up to 6 atm, has little or no effect on the volume of the gas space of the cuttlebone and the density of the cuttlefish will not change appreciably with change in depth.

The cuttlebone is very strong and can withstand high external pressures.

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THE DISTRIBUTION OF GAS AND LIQUID WITHIN THE CUTTLEBONE

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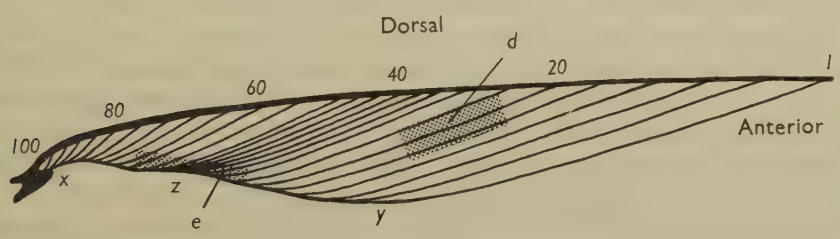
(Plates I and II and Text-figs. 1–7)

We have previously given an account of the general properties of the cuttlebone and the way in which the cuttlefish uses it as a variable buoyancy tank (Denton & Gilpin-Brown, 1961*a*, *b*; Denton, Gilpin-Brown & Howarth, 1961). Here we shall describe how the liquid and gas which the cuttlebone contains are distributed amongst its hundred or so chambers, and attempt to interpret this distribution in terms of the behaviour of the cuttlefish.



Text-fig. 1. Diagram of a cuttlebone (condition in winter) in which only every fourth lamella has been drawn. The true numbers of the chambers are those given along the dorsal surface of the bone. The siphuncular surface is marked *xy*, and it can be seen that the inflexion, *z*, is associated with a number of thin chambers of small volume. The letters *d* and *e* refer to the experiment described on p. 368.

The chambers of the cuttlebone will be numbered from the newest to the oldest chamber (Text-fig. 1). Chamber no. 1 is the incompletely formed chamber lying ventrally and anteriorly; its volume in an adult cuttlefish is between 1 and 2 ml. The oldest, i.e. the first-formed chamber, is approximately no. 100 and its volume is less than 10^{-3} ml. In general the volume of a chamber decreases with increasing number, but on the siphuncular surface of a cuttlebone (*xy*) a marked inflexion can be seen (this is shown by the letter *z* in Pl. 1 and Text-fig. 1), and associated with this inflexion there are a number of very thin chambers of small volume. Despite this complication it is quite clear that the newer chambers, having the larger volumes, are those most important in determining the buoyancy of the cuttlefish. The older chambers, although of small volume, have a disproportionate effect on the

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posture of the cuttlefish for, since they lie well towards the posterior end of the animal, they will, when gas-filled, tend to make the cuttlefish float tail upwards.

It seems possible that the 'pumping out' of a newly formed chamber might be achieved by a mechanism different from that used to control the amount of liquid in the older ones. The siphuncular walls of the newest few chambers are whiter and more silvery in appearance than the corresponding walls of the older chambers. It was found moreover (Denton & Gilpin-Brown, 1961 *a*) that these few are the only chambers which do not lose liquid when the external pressure is reduced. Two simple explanations of this may be suggested:

(i) Liquid is not initially pumped out through the siphuncular wall of a chamber but through the broad ventral wall separating the first and second chambers. The siphuncular walls could, on this hypothesis, be initially impermeable to liquids but become permeable later when six or so more chambers have been added.

(ii) The siphuncular walls of the newest chambers are permeable but the siphuncular ends of these chambers do not contain liquid.

It is shown that the second of these possibilities is the correct one.

The second chamber is the one which is being 'pumped out' for the first time. It is known that liquid is extracted through the siphuncular walls of the other chambers. If, therefore, liquid in the second chamber were found at its siphuncular end, it would be fairly clear that it was being pumped out through the permeable siphuncular wall. There would have to be some differences, e.g. in wettability and dimensions, between the different parts of this chamber to ensure both that the initial 'bubble' is formed away from the siphuncular end and that the column of liquid within the chamber is not completely broken during the pumping out. However, liquid, when present at all in the second chamber, was rarely found at its siphuncular end. The second chamber was indeed the only one in which liquid was not so localized. The following explanations of this may be given:

(i) Liquid is initially extracted through the siphuncular wall but there is no adequate mechanism to ensure that the column of liquid stretching inwards from the siphuncular wall is not broken. The liquid left behind is, however, little by little shaken down to this wall and then extracted.[1]

(ii) Some liquid is extracted through the broad ventral wall of the chamber. Arguing againt this hypothesis is the fact that the living epithelium covering the ventral surface is separated from the wall of the second chamber by the liquid-filled incomplete parts of the first.

(iii) Liquid is first taken from the siphuncular end of the chamber and water then evaporates from the deeper wet parts of the chamber on to the siphuncular surface and is there taken away. The obvious difficulty of this hypothesis is that a progressively stronger salt solution will be left in the deeper parts of the cuttlebone and evaporation will be brought to a stop. We might keep this explanation

[1] Water might be transported by living cells but no such cells could be found within the chambers.

if: either (*a*) the liquid in chamber 1 were not a salt solution but contained organic substances which were later condensed into the solid parts of the cuttlebone, leaving behind pure water which could be removed by evaporation; or (*b*) the salts in the liquid were themselves condensed into the solid structure of the bone leaving pure water behind.

Here it is shown that the water in the first chamber contains sodium and potassium in concentrations close to those of sea water and that these ions are not left behind when the water is initially removed. Since the salts and water leave the newly formed chamber together and, since they are not pushed out by gas pressure, it seems likely that liquid is extracted osmotically. The possibilities listed under (iii) above may therefore be excluded.

It has previously been shown (Denton & Gilpin-Brown, 1961*a*) that when the cuttlefish changes its density by increasing or decreasing the gas space within its cuttlebone the mass of gas within the cuttlebone remains approximately constant. This has been explained in terms of the osmotic movement of liquid and the slowness of diffusion of gases into and out of the cuttlebone. On this hypothesis we should expect that the pressure of gas would be particularly low in a newly pumped chamber. This is shown to be the case. An estimate of the rate of diffusion of nitrogen (the principal gas in the cuttlebone) into and out of the cuttlebone is made on the basis of an experiment in which the rate of entry of carbon dioxide was studied.

METHODS AND RESULTS

The permeability of the siphuncular wall

The following experiment was designed to find out whether or not the siphuncular walls of the newest chambers are permeable to liquid.

Two cuttlebones were taken and the membranes covering their siphuncular surfaces were wiped off. One was placed under sea water for about 2 h. It was then gently dried on a cloth and placed under reduced pressure. Liquid which had soaked into the cuttlebone now came freely from the siphuncular surface, even from the newest chambers (apart from the first incomplete chamber). The second cuttlebone was placed for 30 min in sea water under a pressure of +5 atm. When the cuttlebone was brought back to atmospheric pressure, liquid was observed to flow freely from all the newest chambers except the first. These two experiments demonstrate that in all the chambers, except perhaps the newest of all, which is incomplete, the siphuncular surface is freely permeable to water.

Sodium and potassium in different parts of the cuttlebone

An experiment was made to decide between the various mechanisms suggested for the initial pumping out of a newly formed chamber. The following parts of a fresh cuttlebone were scraped off, each into a separate weighing

bottle: (*a*) the 1st chamber; (*b*) the 2nd chamber; (*c*) the 3rd chamber; (*d*) the anterior regions of some of the older chambers (around the 20th), see Text-fig. 1; (*e*) the posterior regions close to and including the siphuncular surface, of a number of chambers older than 20 (Text-fig. 1). Great care was taken during the scraping to keep the lids on the weighing bottles as much as possible to avoid losses of water by evaporation from the material already collected.

Each of the samples in the weighing bottles was then treated successively as follows: (i) weighed; (ii) extracted with distilled water, and the sodium and potassium content of the extract measured using a flame photometer—*Extract* 1; (iii) dried at 110° C to constant weight; (iv) ashed in a silica crucible at 650° C to constant weight; (v) the ash extracted with distilled water and after the calcium in the extract had been precipitated with ammonium oxalate the sodium and potassium contents were measured—*Extract* 2; and (vi) after water extraction, the ash dissolved in strong hydrochloric acid which was then neutralized with ammonium hydroxide, the calcium precipitated with ammonium oxalate, and the total sodium and potassium in the residual solution found—*Extract* 3.

TABLE 1. ANALYSES OF SUCCESSIVE CHAMBERS

Sample ...		*a*	*b*	*c*	*d*	*e*
Dry weight (g)		0·282	0·266	0·325	0·356	0·323
Weight water / Dry weight		4·27	0·46	0·015	0·024	2·67
Ash weight / Dry weight		0·54	0·53	—	0·54	0·55
Sodium (mg) / Dry weight	1st extract	45·0	6·7	4·9	4·3	27·0
	2nd extract	5·3	4·1	—	4·3	3·3
	3rd extract	0·9	1·0	—	2·0	1·5
Total sodium (mg) / Dry weight		51	12	—	11	32
Bound sodium (mg) / Dry weight		6	5	—	6	5

For explanations of the letters *a* to *e*, see text.

The results of this experiment are summarized in Table 1. The weight of potassium in the extracts was always about 5% of the sodium (in sea water it is about 4%). Three conclusions can be drawn from the experiment.

(1) The siphuncular ends of the older chambers (sample *e*) can be seen to contain much water, whilst the anterior ends (sample *d*) contain very little. This might have been expected, for we have previously found that liquid exchanges in the cuttlebone are made across its siphuncular surface.

(2) All the samples of cuttlebone contained approximately the same proportion of 'bound' sodium and potassium. Here by 'bound' we mean the sodium and potassium which was only extractable after ashing. This amounted to about 10% of the sodium in the first chamber. Since the solid parts account

for about 10% of the total volume of a chamber[1] the amount of sodium trapped in the formation of the solid parts is approximately equal to the sodium in an equivalent volume of sea water.

(3) During the initial pumping out of a newly formed chamber almost 90% of the 'free' sodium and potassium which the chamber initially contained is removed with the water. If we compute the ratio:

$$\frac{\text{Free sodium (mg)}}{\text{weight of water lost on drying (g)}}$$

for the samples (*a*) to (*e*) we obtain the values: 10, 14, 32, 18, 10 respectively (sea water has a corresponding value of 11). The sodium concentration is appreciably higher in the third chamber (*c*) and in the anterior regions of the older chambers (*d*), and this might suggest that water is extracted actively. However, the amounts of water in samples (*c*) and (*d*) were extremely small, 15 and 24 mg respectively, and a small amount of evaporation during the collection of these samples may have taken place. These figures in no way invalidate the conclusion that the salt and water in a new chamber are largely removed together.

The distribution of liquid in the cuttlebone

Three methods were used to determine the distribution of liquid in a cuttlebone.

(1) A cuttlebone was cut sagitally into two halves and one half was placed cut side downwards in a tray containing a small depth of a solution of Sudan Black in xylene. This solution is sucked by capillary action into the dry regions of the bone but it does not penetrate into the wet regions. After about half an hour the bone was removed and examined. The black regions indicated those parts of the bone containing no water, the white regions those parts containing water.

(2) A longitudinal section about $\frac{1}{2}$ cm thick was cut from a cuttlebone and examined by transmitted light under a dissecting microscope. The regions containing water appeared lighter than those which were dry. The wet parts could be marked directly on the bone.

(3) The lamellae of the cuttlebone were scraped off one at a time and places where liquid was seen were marked on the outside wall of the succeeding chamber.[2] We could distinguish (*a*) regions where no liquid could be seen, (*b*) regions in which liquid was visible, and (*c*) regions in which liquid freely welled up on scraping.

[1] The figure of 10% was found by an experiment on the chambered parts of the bone.

[2] The newest chambers contain gas under relatively low pressure and it is possible that on puncturing a chamber air will rush in and perhaps displace liquid from its natural position. Usually, but not invariably, the newest chambers were punctured half way along their lateral margin.

Each chamber is subdivided by about six thin chitinous membranes running parallel to the main walls of the chamber and examination of the distribution of liquid within a chamber, using method 2 above, showed that liquid extends from the siphuncular end by differing amounts in the different subchambers. By combining methods 2 and 3 on the same cuttlebone it was shown that condition (*c*) corresponds to at least two of the seven or so subchambers being full of liquid.

Each method has some special advantage; the first is the quickest, the second allows the distribution of liquid within the subchambers to be seen, and the third ensures that the whole extent of each chamber is examined. All three methods gave the same general results. The first chamber, which is incomplete, is always full of liquid. The succeeding eight or so chambers are usually dry, whilst in the remaining chambers the liquid is all localized at the siphuncular ends of the chambers. The oldest chambers (i.e. those older than the thin chambers associated with the inflexion in the siphuncular surface) are usually very wet, and often almost completely filled with liquid. These chambers are only emptied when the bone becomes of exceptionally low density. In all chambers, apart from the first two, liquid, when present, increased progressively towards the siphuncular end. The siphuncular ends of the chambers were very wet indeed.

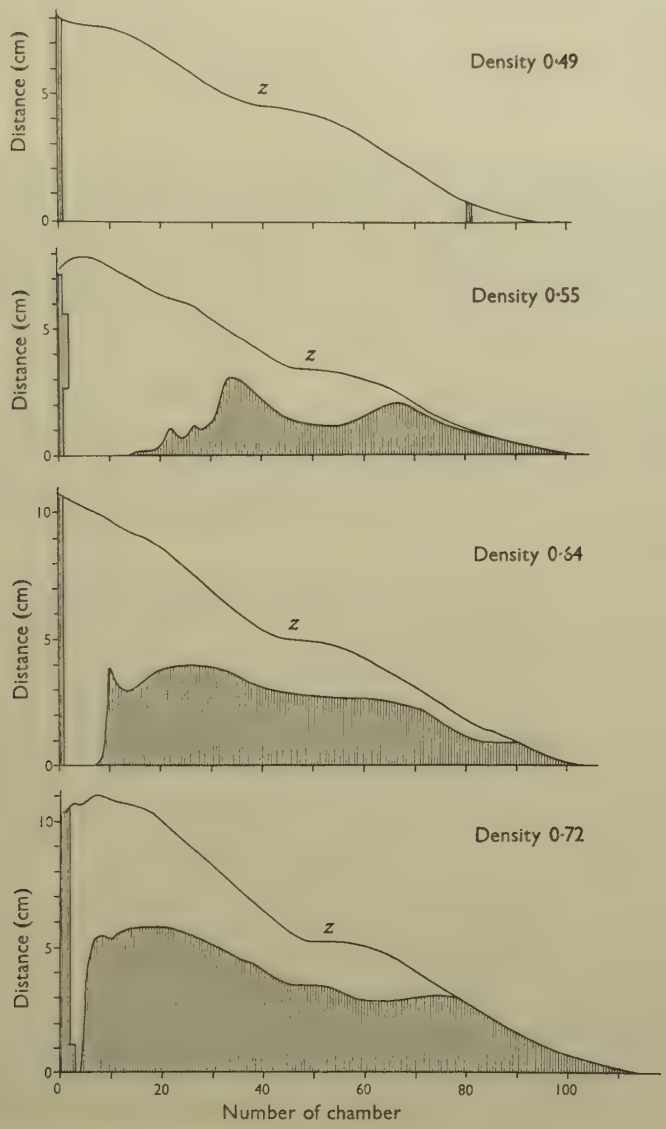
The most complete results are those given by the third method. This was applied to cuttlebones chosen to cover a large range of densities. The results for four of these cuttlebones are given in Text-fig. 2 where two quantities are plotted as ordinate; first the length of the chamber in the midline of the cuttlebone, and secondly that part of the chamber which contained liquid in sufficient quantity that it welled up on scraping (criterion (*c*), above).

The pressure of gas within individual chambers of the cuttlebone

The pressures inside the individual chambers were studied in the following two experiments.

Experiment A

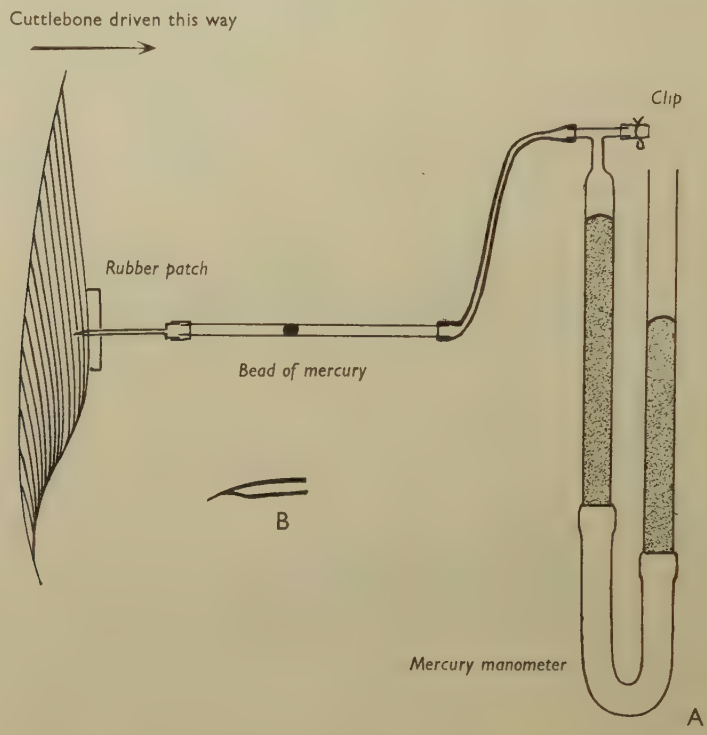
Freshly dissected cuttlebones were placed under sea water containing *Sepia* ink. The chambers were then opened under ink by slowly drawing a sharp edge along the centre of the siphuncular surface, i.e. along the line *xy* of Text-fig. 1. The bones were left for about an hour under ink and then taken out, dried gently and sawn up. Sections of such a bone are shown in Pl. 1. It can be seen that ink has been drawn much more extensively into the newest chambers than into the others. The newest chambers are indeed almost completely filled with ink and must, before puncturing, have contained gas at very low pressures. Practically no ink has penetrated the older chambers (*zx*); this is because these chambers are already almost full of water.



Text-fig. 2. Graphs showing the distribution of liquid in the chambers of four cuttlebones of widely differing densities. The ordinate represents distance along a chamber measured from the siphuncular surface, which therefore corresponds to the base line. The inflexion, *z*, marks the thin chambers of very small volume (see Text-fig. 1). The regions containing liquid (category of (3) *c*, p. 369) are shown by the hatched areas. This category is such that at least two of the seven or so subchambers making up a chamber contain liquid. The number of subchambers filled with liquid increases progressively towards the siphuncular end.

Experiment B

The volumes of the gas spaces and the pressures of gas within individual chambers were determined quantitatively using the apparatus shown in Text-fig. 3.

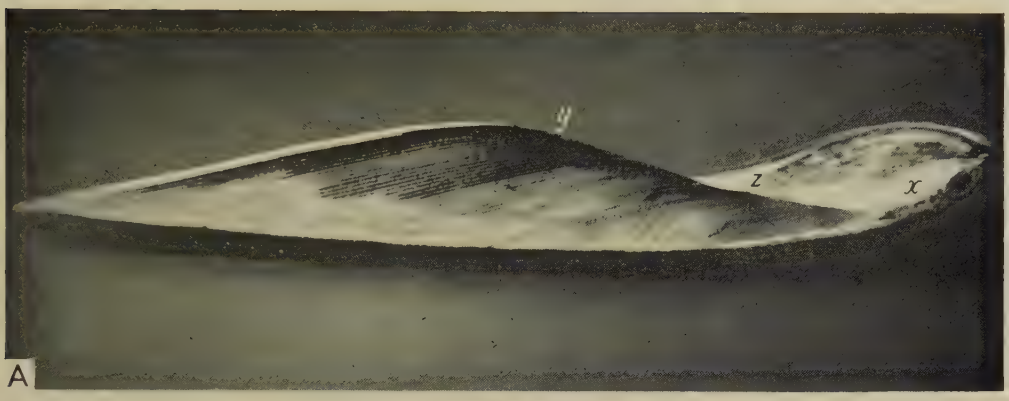


Text-fig. 3. A, Diagram (not to scale) of apparatus used to determine the gas pressures within the cuttlebone. The cuttlebone is shown in cross-section and three chambers have already been punctured. B, Diagram of the tip of the needle of the hypodermic; it has been bent over to avoid the needle blocking with material from tne walls of the cuttlebone.

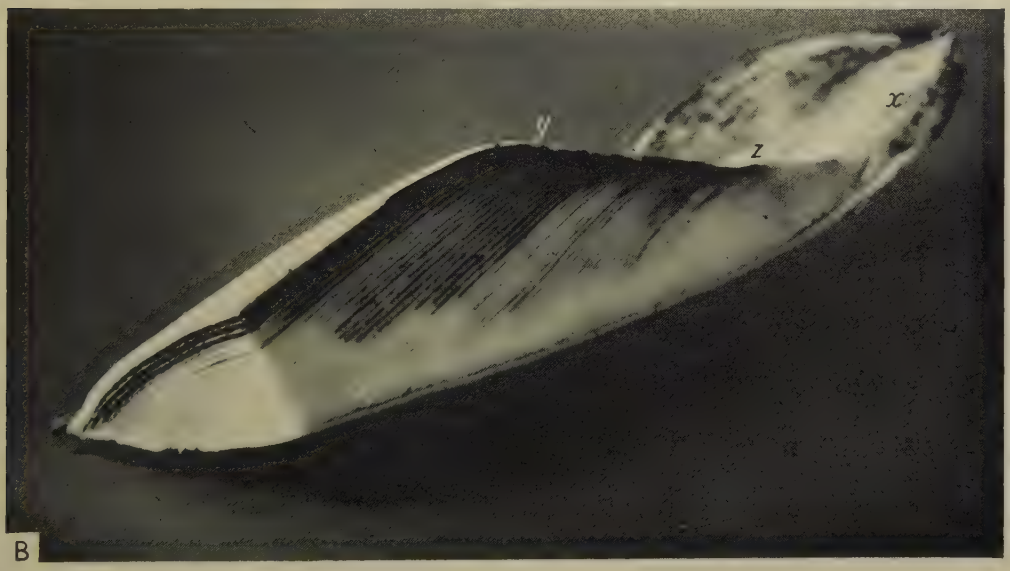
A rubber patch was stuck on to the anterior ventral surface of the cuttlebone. Two rubber bands were passed round the bone and the patch to make quite sure that it did not slip. The bone was held vertically in a clamp carried on a horizontally lying Palmer adjustable stand.

Explanation of Plate I

This cuttlebone has had its chambers punctured under sea water containing *Sepia* ink by a scratch along its siphuncular surface (*xy*). A, A longitudinal section; B, a section showing the same cuttlebone after cutting away its anterior end to allow the lateral penetration of ink to be seen. Ink has been extensively drawn into the newer chambers which contain gas under low pressures. In this plate the cuttlebone is shown ventral side upwards.

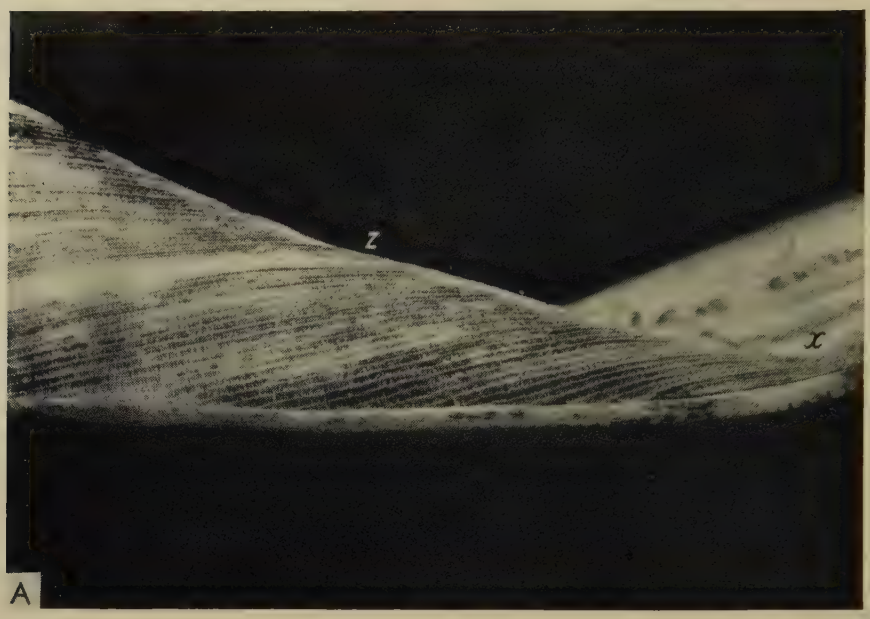


A



B

(*Facing p.* 372)


z
x
A

z
x
B

A 1 ml. graduated pipette was held horizontally in a second stand and this contained somewhere along its length a bead of mercury. The tapered end of the pipette was connected by a short piece of rubber tubing to a no. 14 hypodermic needle whose point had been bent as is shown in the detail B of Text-fig. 3. The other end of the pipette was connected by narrow bore tubing to a mercury manometer made of wide bore tubing. By raising and lowering one arm of the manometer the pressure of gas in the pipette could be varied in a known way.

The bead of mercury was brought close to the end of the pipette farthest from the open needle. The bone was now moved slowly on to the hypodermic needle so that this slowly punctured the bone through the rubber patch. (The tip of the needle had been bent so as not to be blocked by a neat core taken from the material of the cuttlebone.) The bead of mercury was watched carefully. Suddenly a marked displacement of this bead towards the needle was seen; the puncturing was then stopped. The needle had clearly penetrated into some gas space containing gas at less than atmospheric pressure.

Using the mercury manometer the gas pressure was varied and the positions of the mercury bead in the pipette found for various pressures.

These measurements finished, the cuttlebone was slowly driven farther on to the needle until a second sudden displacement of the mercury bead was observed. The puncturing was again stopped and volume and pressure measurements again made. This procedure was repeated a number of times, the needle being pushed farther and farther into the bone. Finally the needle was withdrawn. After finding the volume and density of the bone, this was sawn up and shaved gently with a scalpel to expose the path of the needle. The number of chambers punctured could then be counted. This number was equal to one more than the number of steps in pressure on puncturing. Since the newest chamber is incompletely formed and full of liquid this means that each change in displacement of the mercury bead corresponded to the puncturing of one chamber of the cuttlebone.

Text-fig. 4 shows a plot of volume change against the reciprocal of the pressure for the gas in the second chamber of a cuttlebone together with some gas in the measuring pipette. It can be seen that the points fall on a straight

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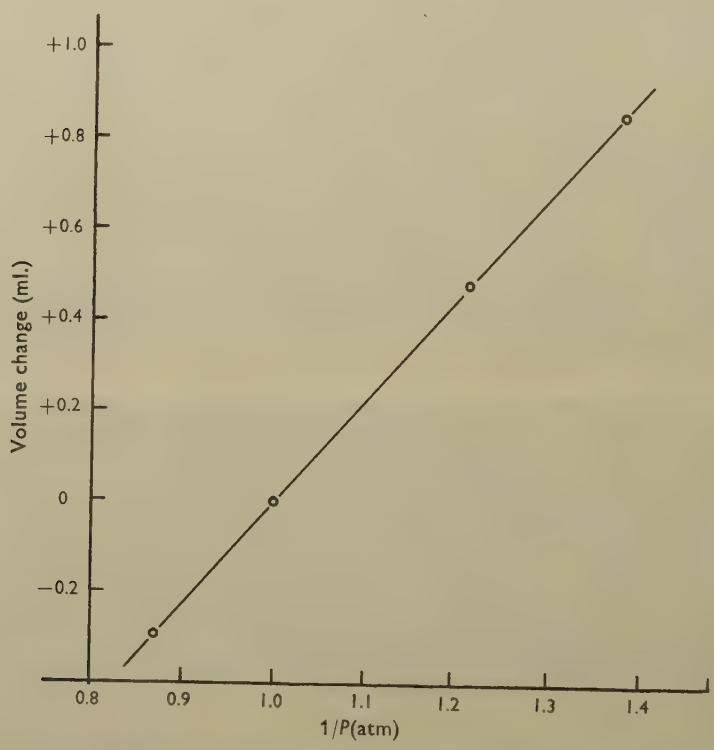
Explanation of Plate II

A. A cuttlefish was kept in the dark for 2 days. Its cuttlebone which had then a low density (0·53) was punctured, under sea water containing *Sepia* ink, by a scratch along the siphuncular surface. The ink has penetrated very far into the older chambers (marked *zx*) showing that the pressure of gas within these chambers must have been very low before puncturing.

B. Similar to A except that the cuttlefish had not been kept in the dark. The cuttlebone's density was about 0·62. Ink has not penetrated into the older chambers because these were already almost full of liquid. In this plate the cuttlebones are shown ventral side upwards.

line, as Boyle's law would predict. This is true for the individual chambers of the cuttlebone. The volumes indicated by the application of Boyle's law correspond closely to estimates made of the volumes of the chambers from their physical dimensions.

It is now possible using Boyle's law to calculate the volume of the individual chambers and the pressures of gas within them before puncturing. The calculation which gives the volume of the gas space and the pressure of gas in the first chamber is given as an example.



Text-fig. 4. Graph showing that the gas within the second chamber obeys Boyle's law. A straight line is given on plotting 1/pressure against volume change.

Before puncturing let V_1 be the volume of the gas space within the first chamber, and P_1 the pressure of gas in this chamber, and let V_A be the volume of gas between the tip of the needle and the mercury bead in the pipette, and P_A the pressure of gas within the pipette.

After puncturing the first chamber let V_b be the volume of the gas space between the tip of the needle and the mercury bead at pressure P_b and let V_c

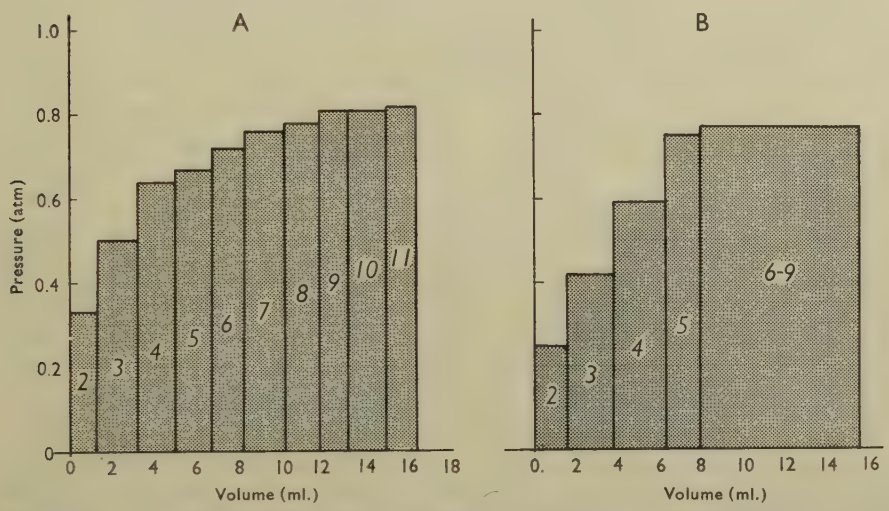
be a corresponding volume at the pressure P_c. Using Boyle's law, which was shown to be valid here, we may now write the equation:

$$(V_1+V_b)P_b = (V_1+V_c)P_c,$$

and from this we can find V_1. We can now substitute the value for V_1 in the equation:

$$P_1V_1+P_aV_a = (V_1+V_b)P_b = (V_1+V_c)P_c,$$

and so find P_1. This kind of calculation we can repeat for each successive puncturing and so find the volumes of gas spaces and the pressures within successive chambers.[1]



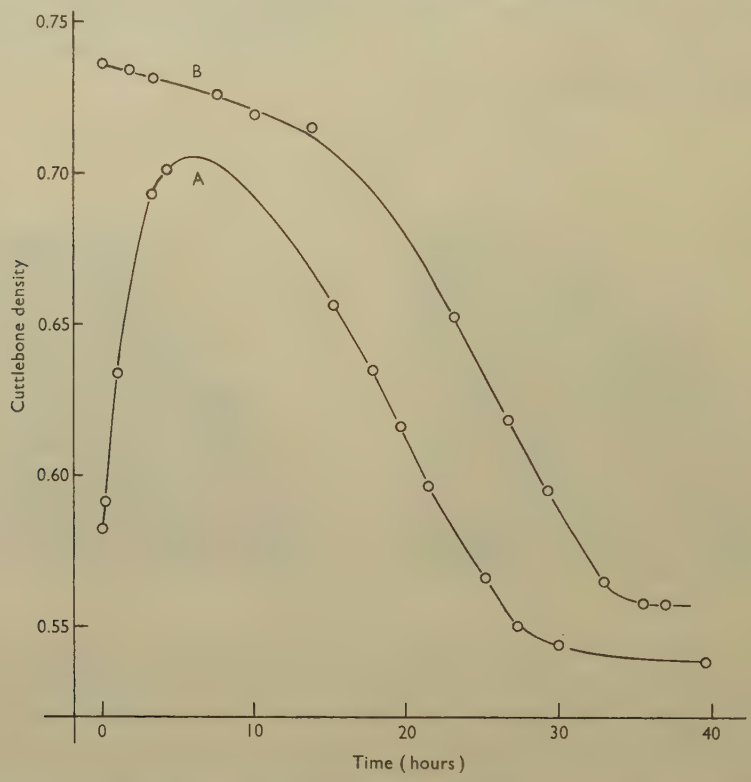
Text-fig. 5. Graphs showing as abscissa the cumulative volumes of the chambers of the cuttlebone starting from the most recently formed gas-filled chamber (chamber 2). The ordinate is pressure of gas within the individual chambers. A is for a cuttlebone in which the newest 10 gas-filled chambers were punctured in turn. B is for a cuttlebone in which, after puncturing the first four gas-filled chambers individually, several were deliberately punctured together.

Text-fig. 5 shows results obtained in this way on two cuttlebones. Here the cumulative volumes of the chambers punctured are plotted against the pressures of gas within these chambers before puncturing. It can be seen that the pressure of gas is much lower in the newest chambers than in the somewhat older ones and that by the time we reach about the tenth chamber the pressures are close to the figure of about 0·8 atm found as the average pressure of gas within the cuttlebone (Denton & Gilpin-Brown, 1961*a*).

[1] The 'dead space' between the tip of the needle and the end of the pipette can be readily found. The needle is closed by pushing it into a rubber bung and the readings of the mercury bead along the pipette are measured for various pressures. The volume of 'dead space' and pipette is found by using Boyle's law.

The slowness of diffusion of gases into the cuttlebone

To find at what rates gases within the cuttlebone will come to equilibrium with gases in the tissues around the cuttlebone a freshly dissected cuttlebone (Text-fig. 6, A) was placed in sea water saturated with carbon dioxide and through which carbon dioxide was being bubbled. Its changes of density with time were then found. A second cuttlebone (B) was soaked in sea water until



Text-fig. 6. An experiment to determine the rate of diffusion of CO_2 into the cuttlebone. A, A freshly dissected cuttlebone was placed in sea water saturated with CO_2. B, A cuttlebone was initially soaked in sea water until it reached constant density and then at time 0 hrs it was transferred to sea water saturated with CO_2.

it attained constant density (Denton & Gilpin-Brown, 1961*a*, fig. 11) and then transferred to carbon dioxide saturated sea water. Carbon dioxide was chosen because it is much more soluble than either nitrogen or oxygen in sea water and will therefore come more quickly to equilibrium.

The results of this experiment are shown in Text-fig. 6. The curve A appears rather complicated but its form can be simply explained in terms of our knowledge of the cuttlebone. At first (as with the cuttlebones of figure 11

of Denton & Gilpin-Brown, 1961*a*) water was pushed into the cuttlebone by the difference in pressure between the inside and outside of the bone. As this process slowed down the cuttlebone density then began to decrease, for carbon dioxide was all the time diffusing into the cuttlebone much more quickly than the gases inside could diffuse out. The gas pressure inside the bone therefore rose and water was expelled through the siphuncular surface. This process became increasingly fast as more and more liquid was expelled and the diffusion path for carbon dioxide became shorter; finally, when all the liquid available had been expelled from the cuttlebone its density remained almost constant, but the pressure of gas in the bone continued to rise. At the end of the period of soaking the cuttlebone was punctured and a stream of gas bubbles came from the hole, showing that the pressure of gas was above atmospheric.[1] The curve B shows very similar results, but here the first phase, in which sea water is taken up, has been avoided by the initial soaking in sea water.

For convenience the numerical results of this experiment are given during the course of the discussion.

The slowness of gaseous diffusion into a cuttlebone was also shown by an experiment in which a cuttlefish was kept in the dark for 2 days so that its cuttlebone became of very low density, 0·53. This cuttlebone was placed under *Sepia* ink and all its chambers were punctured in turn by a scratch along the siphuncular surface. It can be seen (Pl. II) that the ink has penetrated very deeply into the older chambers which are usually filled with liquid, but which in this cuttlebone will have just been pumped out. We thus see that the pressure of gas within these chambers was very low, and we have reproduced here the low pressures found in a newly formed chamber. In this case, of course, the liquid has certainly been pumped out from the siphuncular ends of the chambers, but we are not certain yet that this is the case for the newest chambers.

Capillarity

It seemed possible that surface tension forces (capillarity) might be used to ensure that liquid remained at the narrow siphuncular ends of the chambers, and we have already seen that a xylene solution is quickly drawn by capillarity into the narrow gas-filled space of the bone.

Cuttlebones, both fresh and dried, were sawn sagitally into two halves, and half cuttlebones were placed cut side downwards in a trough containing a shallow depth of a solution of aniline blue in water. Those parts of the chambers far away from the solution were punctured to make sure that the liquid could enter freely.

The watery solution of aniline blue was not taken up at all.

[1] This experiment resembles that in which a porous pot filled with air is placed in an atmosphere of hydrogen at the same pressure. The hydrogen diffuses into the pot much more quickly than the air diffuses out, and the gas pressure inside the pot therefore rises.

It therefore seems that the inside walls of the chambers of the cuttlebone are not very 'wettable' and we have no evidence that surface forces are used to localize liquid at the siphuncular ends of the chambers. This is in agreement with earlier conclusions (Denton & Gilpin-Brown, 1961*a*).

DISCUSSION

The slowness of equilibration of gases in the cuttlebone is partly because the distance for diffusion is great, but also because the large gas space into which the gases diffuse will hold, at a given pressure, a great deal more oxygen and nitrogen than will an equivalent volume of water. We can get some idea of how slow equilibration will be from the experiment on the uptake of carbon dioxide. Here, when the densities of the cuttlebones were about 0·6, the rates of uptake of carbon dioxide were such that the times for the cuttlebones to move only half way towards equilibrium would have been about one day.

Now, for a given partial pressure difference, nitrogen diffuses in water at only about $\frac{1}{40}$th the rate at which carbon dioxide diffuses[1] (Hufner, cited by Krogh, 1919) and so the half time of equilibrium for nitrogen would not be 1 but 40 days. This explains why, when the cuttlefish changes the volume of liquid within the cuttlebone, the mass of gas within the chambers remains almost constant for a long time. It also explains why the pressure of gas within the newest chambers is so low for these will have been formed recently and insufficient time will have elapsed for equilibrium to be attained. The distribution of pressures in the newest chambers (Text-fig. 4, p. 374) shows that it is not until we reach about the 9th chamber that equilibrium is approximately attained. Since the chambers are probably only laid down at a rate of three to five a month, this probably represents a period of 2 months, a very long time when we remember that these chambers are almost dry and the diffusion path for gases is relatively short.

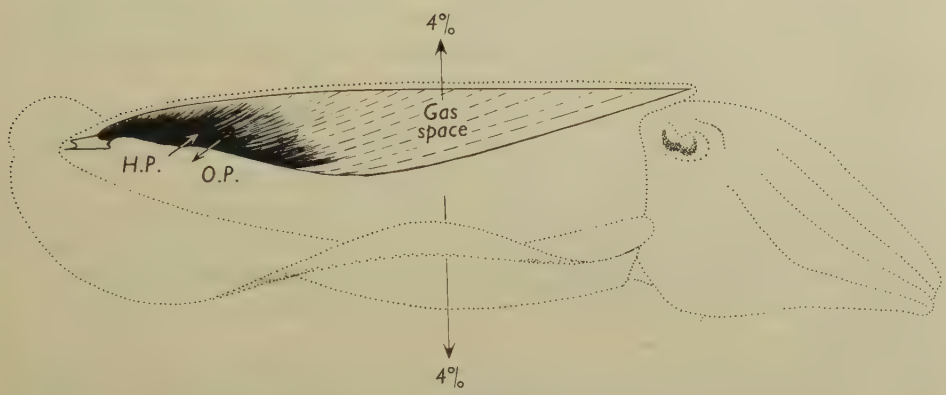
Since differences in pressure of gas as well as differences in liquid content are maintained between neighbouring chambers, the principal walls of the chambers must be impermeable to both liquids and gases.

The pattern of water distribution in the cuttlebone has been shown to be a fairly simple one, and the following account summarizes the results both of the experiments which have been described here and many other observations on the cuttlebone. In all cuttlebones the newest chamber (no. 1) is incomplete and filled with liquid. The second chamber, although often dry, may contain some liquid but this is rarely localized at the siphuncular end. In cuttlebones of density around 0·6 the oldest and most posterior chambers

[1] The difference in diffusion rates arises principally because carbon dioxide is much more soluble than nitrogen in water so that for a given partial pressure difference there are many more molecules of carbon dioxide than nitrogen available for diffusion. Since the ratio of solubilities of nitrogen and carbon dioxide does not differ very much between pure water and sea water (Harvey, 1955) we may use the value of $\frac{1}{40}$th here.

are almost completely filled with liquid; whilst the second to about the 10th chambers, that is those most newly formed, are dry. In very light cuttlebones (densities close to 0·5) there is very little liquid visible anywhere, and even the oldest chambers have been pumped dry. In very dense cuttlebones (densities close to 0·7) almost all the chambers, including most of those newly formed, contain liquid.

The distribution of liquid in cuttlebones of densities around 0·62, i.e. densities close to the cuttlebone density which will make an animal neutrally buoyant is a rational one. The older chambers, which are very posterior, are kept fairly full of liquid, and the newer chambers, which lie more centrally along the length of the animal (Text-fig. 7), are kept dry. This means that



Text-fig. 7. Diagram summarizing our knowledge of the cuttlebone. The cuttlebone here represented has a density of about 0·6. Liquid within the cuttlebone is marked black. It can be seen that the oldest and most posterior chambers are almost full of liquid. If they were filled with gas, this would tend to tip the tail of the animal upwards. The newest 10 or so complete chambers, which lie centrally along the length of the animal, are completely filled with gas. These chambers can give buoyancy without disturbing the normal posture of the animal.

The hydrostatic pressure (H.P.) of the sea is balanced by an osmotic pressure (O.P.) between cuttlebone liquid and the blood. In sea water the cuttlebone gives a net lift of 4% of the animal's weight in air and thus balances the excess weight of the rest of the animal. *Note.* The black markings in this figure have quite a different meaning from those of Plates I and II.

when the animal is approximately horizontal its centre of gravity is brought under its centre of buoyancy so that it can remain in this posture without effort. The cuttlefish can, however, both pump out the older chambers and let liquid into the newer ones. Since the main body of liquid lies at the siphuncular, i.e. posterior, ends of the chambers, when water is pumped out of the cuttlebone this will tend to bring the tail up. Having the tail slightly higher than the head is probably a good posture for the cuttlefish when it hunts over the sea bottom. It is certainly the posture which a cuttlefish takes in an aquarium tank as it prepares to attack a crab or prawn.

We wish to thank Mr G. A. Battin for advice and help with the illustrations in this and the previous communications.

SUMMARY

The newest chamber of a cuttlebone is always incomplete and full of a watery solution. This contains sodium and potassium in concentrations close to those of sea water.

The second newest chamber contains a gas space and sometimes contains liquid. This liquid is rarely localized at the siphuncular end of the chamber. It is not certain that liquid is initially extracted through the siphuncular wall of the chamber, although this is possible.

When liquid is pumped out of a newly formed chamber the salt which it initially contains is not left behind, either free or bound into the structure of the bone.

The 3rd to about the 10th newest chambers usually contain no visible liquid. But their siphuncular walls are permeable to liquid and liquid does enter these chambers when the cuttlebone becomes very dense.

At cuttlebone densities around 0·6, i.e. close to values which will make the cuttlefish neutrally buoyant, the older and more posterior chambers are almost full of liquid. This liquid can, however, be pumped out. When a cuttlebone has a density around 0·5 very little liquid can be seen in any of its chambers.

The pressure of gas within a newly 'pumped out' chamber is very low, but by the time a chamber has become the ninth newest the pressure of gas is close to the average value for the whole cuttlebone, i.e. about 0·8 atm.

The low pressure of gas found in the newest chambers is explained by the slowness with which gas diffuses into a space created by the active removal of liquid.

It is estimated, from an experiment on the diffusion of carbon dioxide into the cuttlebone, that it would take over a month following a change in density of the bone, for the nitrogen in the bone to go half way to equilibrium with the nitrogen in the tissues. This explains why, when the cuttlefish changes the density of its cutlebone, the mass of gas within the cuttlebone remains almost constant.

The normal distribution of liquid within the cuttlebone is such that the cuttlefish can easily remain with its body horizontal in the sea. When the cuttlebone is made less dense, the change in the distribution of liquid will tend to tip the tail of the animal upwards.

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THE REPRODUCTION AND MAINTENANCE OF *POLYDORA CILIATA* (JOHNST.) AT WHITSTABLE

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(Text-figs. 1–8)

An abundant population of *Polydora ciliata* (Johnst.) is found living in the London Clay exposed at L.W.S.T. on the beach at Whitstable. The species makes its burrows in the clay kept free of loose superficial material, and is not normally found in mussel beds or where there is accumulated silt. In large areas of the clay *Polydora* is apparently free of any direct competition, the only other members of the in-fauna being a few *Corophium volutator* and the venerid mollusc *Petricola pholadiformis* (see Newell, 1954). The clay therefore offers an ideal environment in which to estimate the effects of natural hazards on a single species.

Some information is available on the time of breeding in records of various marine stations and from plankton hauls containing the larvae. Apart from this, little is known of the life-span, growth rates, fecundity or mortality of the adults or the planktonic larvae.

BREEDING MORPHOLOGY

Development of the germ cells

The considerable literature on the development of polychaetes contains few references to the site of origin of the germ cells. They may arise from the peritoneal covering of the parapodial blood vessels as in *Aphrodite* (Fordham, 1925), or from the ventral coelomic epithelium between the parapodia as in *Nereis* (Dales, 1950). *Arenicola* has a gonad situated behind the nephridium where the germ cells develop before passing into the coelom (Ashworth, 1904). Hempelmann (1931) states that the genital products of the spionids, *Laonice*, *Prionospio* and *Spiophanes* occur between the parapodia but does not mention their place of origin. Of the rest many would seem to be without a structurally differentiated gonad, the sex cells proliferating from the coelomic epithelium.

The gonads of *Polydora ciliata* first become visible in worms of 40–45 segments as a pair of club-shaped bodies projecting into the ventral coelom in

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each of segments 7–10 inclusive. They arise from the medial border of the ventral longitudinal muscle in the middle of the segment (Fig. 1, *g.*). Sections of the female worm in this region show the oocytes lying immediately internal to the ventral glandular epithelium at the base of the parapodia, and beneath the nerve cords and the ventral longitudinal muscle. They are easily recognized by their prominent vesicular nucleus and the granular nature of their cytoplasm. From this position they migrate into the gonads. Here they remain until they are 25–30 μ in diameter and are then released into the

Fig. 1. *Polydora ciliata.* Transverse section through ovary. *g.*, gonad; *mig.oo.*, oocytes migrating into gonad; *mu.c.*, mucus cells; *n.c.*, nerve cord; *v.l.m.*, ventral longitudinal muscle.

coelom by the rupture of the epithelium covering the gonad. From the perivisceral coelom of segments 7–10 they are transported posteriorly and accumulate in the parapodial cavities of segments 17–37. In this region of maturation the oocytes grow from an initial diameter of 30 μ to their maximum of 130 μ shortly before release.

The sperm mother cells, which are not easily distinguished from the oocytes in the early stages, divide to form spermatocytes and spermatids. They are usually found in small discoid bundles known as sperm plates. Mature spermatozoa are 65 μ long with a long tail and sharply pointed acrosome. They break away from the sperm plates shortly before release and lie free in

the coelom. *Polydora ciliata* does not possess spermatophores, though they occur in *Microspio* (Franzen, 1956), and *Pygospio* (Söderström, 1920).

How the transport of the germ cells from the gonad to the region of maturation is achieved remains uncertain. Free oocytes and spermatocytes have not been found between the tenth and seventeenth segments.

Nephridia and genital ducts

Goodrich (1946) describes the Spiomorpha as having mixonephridia. The nephridia of *Polydora ciliata* are found from the eleventh segment to the end of the trunk region (i.e. the segments bearing the branchiae). In segments 11–16 (Fig. 2A) they are small and undifferentiated, having a small ciliated funnel on the intersegmental septum followed by a thin-walled ciliated tube

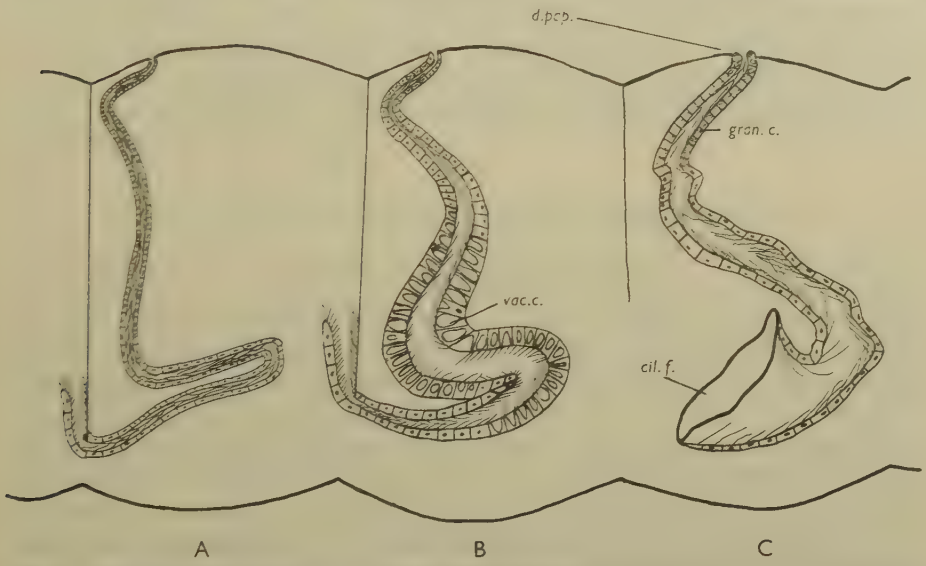

Fig. 2. Nephridia of *P. ciliata*. *cil.f.*, ciliated funnel; *d.pap.*, dorsal papilla; *gran.c.*, granular cells; *vac.c.*, vacuolated cells. A, normal; B, male; C, female.

opening on the dorsal body wall of the following segment. There are a pair of nephridia in each segment. In the region where the germ cells mature the nephridia become much modified, those of the male differing considerably from those of the female, and serving as sperm and oviducts.

The nephridia in the genital storage region of the male (Fig. 2B) undergo an enlargement of the middle region of the ciliated tube. The cell borders of the cells forming the wall of the tube in this region remain ciliated but the cytoplasm contains large vacuoles. The terminal part of the tube passes dorsally, crossing the dorsal body wall transversely to open on a small papilla lying to one side of the mid-line.

The nephridium of the female (Fig. 2c) does not possess this thickened middle region of the male. The ciliated funnel is, however, much enlarged and frequently appears to become detached from the intersegmental membrane, at least in preserved material. The cells of the ascending and transverse regions of the tube are ciliated and contain large numbers of granules giving staining reactions of neutral polysaccharides and glycogen with the PAS technique. Surrounding the opening of the tube on the dorsal body wall is a small group of mucous cells.

Fig. 3. Egg capsules of *P. ciliata* (from Wilson, 1928).

Formation of the egg capsules

The eggs of *P. ciliata* are laid in thin-walled transparent sacs (Fig. 3) attached to the wall of the tube lining the burrow. Each segment in the genital region produces a capsule and these are joined to the capsules of the segments in front and behind. Repeated attempts to observe the formation of the capsules in worms living in glass tubes were unsuccessful. Söderström (1920) claims that the capsules are formed by a secretion from the nephridia, an opinion arrived at by observations of the structure of the egg capsule of *P. ciliata* and the related *Pygospio elegans* Clap. *Pygospio* has a similar egg-laying habit, only each capsule is attached to the wall of the tube by a single filament instead of the two as in *Polydora*. This is interesting as the ducts of the genital nephridia of *Pygospio* are fused in the mid-line dorsally and empty through a single papilla in each segment, whereas in *Polydora* the nephridial aperture is double.

The capsule in *Polydora* is thought to be formed in the following way. The worm presses its dorsal side against the wall of the tube and draws away, leaving an attachment from the pore of each nephridium. Egg expulsion then begins, the secretion discharged from the walls of the nephridium leaving a cavity into which the eggs pass. As the capsule fills and expands, it meets the capsule of the other side and they stick together. In a like manner the capsules stick to those discharged from the neighbouring segments. The secretion, at first thin, hardens rapidly on contact with sea water and becomes less sticky.

This sequence of events accounts for most features of capsule formation satisfactorily. The substance of the capsule is mucoprotein and stains with Alcian blue, but apart from a few small cells at the end of the tube no other cells of the nephridium have similar staining properties. Possibly the granules in the ciliated cells are some kind of premucin body. There are also two broad bands of mucus secreting cells running transversely across the dorsal epithelium in each segment which may supplement the primary capsule secreted by the nephridium.

Fertilization

Since the eggs are surrounded by a capsule, fertilization (in the absence of internal fertilization) must take place by the sperm penetrating the capsule during or after its formation. Females of *P. ciliata* living in glass tubes in the laboratory laid eggs only in the presence of ripe males, never in their absence. When examined the eggs had always cleaved, the earliest embryo found being at the two-cell stage. The spermatozoa are released into the water where they find their way to the burrow of the female and are carried in with the respiratory current.

PERIOD OF BREEDING, SETTLEMENT AND GROWTH

Regular samples were taken from a small area which could be easily recognized at each visit. *Polydora* were extracted from their tubes by placing the clay in a 0·5 % solution of phenol in sea water. In 4–5 h most of the worms had crawled from their tubes and were collected with a pipette. The surface of the clay was then examined under a low-power dissecting microscope and any small worms previously missed were removed.

The number of segments in each worm was counted and this was used as an index of the age of the worm. The growth rate was determined by the increase in the mean number of segments for the sample in each month. Note was kept of the frequency of the sexes and, in later collections, the diameters of five oocytes from the body cavity of each female worm were measured. When both adults and recently metamorphosed larvae were present in the sample, the growth rates were measured by first separating the component groups using arithmetic probability paper in the manner indicated by Harding (1949). This method, which has recently been used by Forster (1959), facilitates the analysis of overlapping distributions and allows the means of the component groups to be estimated. The derived information, allied with the age distribution of the total sample, made it possible to obtain a fairly accurate estimate of the percentage of each component group in the whole sample. The sample mean for each month was checked statistically to determine whether it differed significantly from that of the previous month.

The analyses of the November 1955 and the January 1956 samples (Fig. 4) gave every appearance of a population normally distributed with respect to

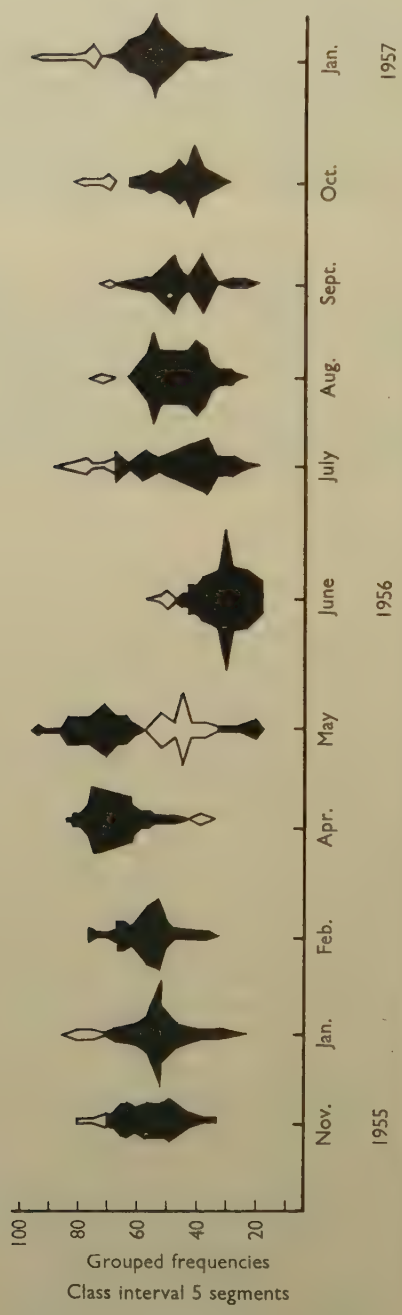

Fig. 4. Monthly distribution of *P. ciliata* according to size. White areas represent the early breeding worms and their young. Black areas represent the later breeders.

age with means of 56·4 and 54·6 segments respectively. In the January sample many of the worms of 70 segments and above were found to have mature oocytes in their coelom and some of the burrows contained egg strings. In the following month, winter temperatures reached their minimum (Fig. 5), and, although the dispersion of the sample is not so great as in the previous month, the mean number of segments (55) did not differ significantly from the mean for November 1955 and January 1956. Many worms in the February sample were found to have mature oocytes in their coelom but still only a few burrows contained egg strings.

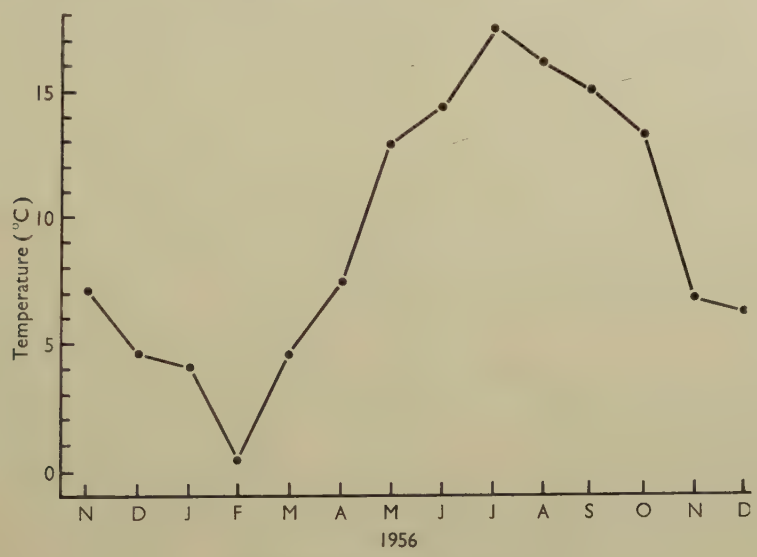

Fig. 5. Sea temperatures at Whitstable in 1956.

The rising temperature of the sea water during March coincided with a rapid increase in growth, and in April the mean for the total population was 65·5 segments. The majority of the worms spawned during this period and egg capsules were to be found in many of the burrows. Larvae of all stages abounded in the inshore plankton and an increase in the smaller size groups of the sample suggested that some settlement of larvae had taken place. Analysis of the sample by means of probability paper showed that it consisted of an adult population and a recently settled larval group with means of 67·1 and 36 segments respectively.

The May sample indicated that considerable larval settlement had taken place (Fig. 4). Upon analysis it was found that the distribution was trimodal, with means for each group at 72·5 segments, 44·3 and 20·8 segments respectively. It is evident that the adult worms of the April sample with a mean of 67·1 segments correspond to the adults of the May sample with a mean of 72·5

segments. Also the juvenile group of the April sample corresponds to the intermediate group of the May sample, intervening growth increasing the mean from 36 to 44·3 segments.

The adults of the April sample occupied about 95 % of the total population, but in May this fell to 51 %, presumably because of the mortality of the adult worms after the larvae had hatched. The larvae metamorphosing in April had completed their settlement by May, increasing from 5 % to 42·5 % of the total population.

The June sample showed that the second larval settlement, begun in May, had passed its maximum and the worms of this group now well established. All worms from the overwintering and breeding stock had died and are not represented in this or in succeeding samples: the distribution was once more

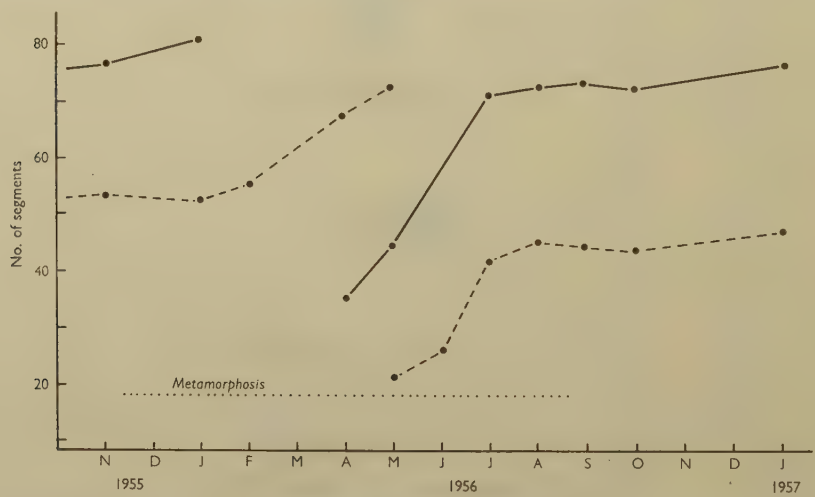

Fig. 6. Growth rates of *P. ciliata* at Whitstable. Continuous line, early-breeding group. Broken line, later-breeding group.

bimodal with two recently settled larval groups with means of 59·5 and 27·8 segments. In the July sample the worms of the first larval settlement had increased their mean size to 70·9 segments, but their proportion in the population had fallen from 42·5 % to 13 %. A few worms of this group had oocytes in the coelom though they were small and not mature. The second group of juvenile worms had grown to a mean size of 41·2 segments and complete the remaining 87 % of the population.

In August growth practically ceased (Fig. 6) with the group means at 71·7 and 42·5 segments respectively. September and October showed no significant increase in the mean size of either group. Many of the larger worms had oocytes, which grew slowly over this period to reach their maximum size in October and November, but the smaller worms of the second larval settlement

were still immature. In October the larger worms formed 8 % of the total population as compared with the 92 % of those with the smaller mean number of segments.

The sample taken in January 1957 showed the same bimodal structure of the population, but in the unusually mild winter with high minimum temperatures in December, growth seems to have been renewed a little earlier than in the previous year. Those worms that overwintered with a mean of 71 segments had now grown to a mean size of 89·6 segments, and were in breeding condition, many of the burrows containing egg capsules with eggs or developing larvae. The second and smaller group of worms had grown to a mean of 49·5 segments and were still immature.

To review the changes taking place over 1956, it is seen that an apparently homogeneous population of *P. ciliata* gave rise to two larval settlements with maxima in April and late May. These groups retained their identity through the summer and the following winter. In January 1957 the worms that had settled in the previous April, and had overwintered with a mean size of 71 segments, were already breeding, whilst the second group, that settled in late May, had yet to reach maturity. In the light of these results it was decided to re-examine the samples of the overwintering stock of 1955–56 to see if they reflected the bimodal condition shown in 1956–57, for, if they did, the two larval settlements would be accounted for.

Analysis of the November 1955 sample revealed that the distribution was in fact bimodal, the two groups having means of 76·5 and 52·5 segments. This discontinuity was also present in the January sample, the more adult group of the November sample had increased its mean to 80·3 segments, whilst the mean for the smaller worms remained unchanged. The proportions of the two groups present in the sample were 90 % for the smaller sized worms and 10 % for the larger ones. It is interesting to note that this forms an extremely close parallel to the proportions of 92 and 8 % in the sample for January 1957.

In February 1956 the distribution of the sample was unimodal, the early breeding worms presumably having died after the larvae had hatched. The remaining worms reached maturity and spawned in late March and early April and their larvae provided the second larval settlement in May.

FECUNDITY

During the months of January to April whilst the worms are breeding, the egg capsules can be extracted entire by carefully breaking open the burrow. Counts were made of the number of capsules in each string and the number of eggs in each capsule. The early-breeding worms were found to have a lower fecundity than the later breeders. In fact, this, and the temporal difference in breeding, form the only apparent difference between them. The early breeders produced on average 17–18 capsules per string with an average content of

11–12 eggs per capsule. This gave an average of just over 201 eggs per female. The later-breeding worms had a considerably higher fecundity, producing an average of 19 egg capsules per string with about 60 eggs in each capsule. This gave an average of 1140 eggs per female. This is in substantial agreement with Hempel (1957) who found that in the North Sea and Baltic, *Polydora ciliata* produced from 12 to 18 capsules each containing approximately 50 eggs.

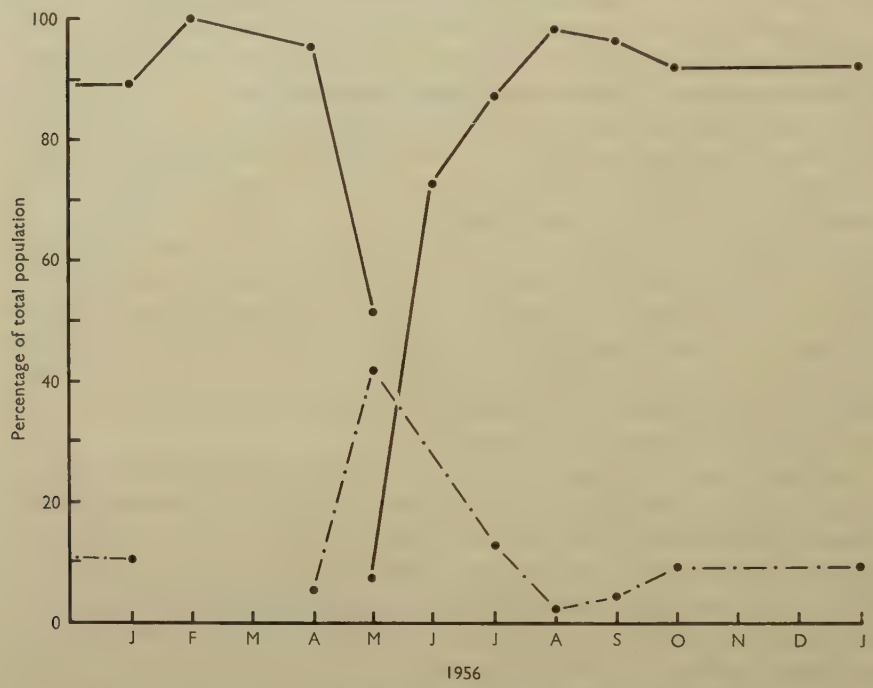

Fig. 7. Population composition of *P. ciliata* at Whitstable in 1956. Continuous line, later breeding group. Broken line, early breeding group.

Söderström (1920) records finding 70–80 eggs in each capsule in egg strings taken from the coast of Sweden, while Wilson (1928) found 15–20 capsules in an egg string, and 15–20 eggs in a capsule at Plymouth.

The figures quoted above include strings containing well developed larvae which were killed in alcohol before puncturing the capsules and counting them. A watch was kept for any undeveloped eggs in order to estimate any effect these might have on the overall fecundity, but none were detected. This observation has received support from Hannerz (private communication) who has examined many egg capsules in the course of his studies on the development of the Spionidae. It would seem that all the eggs develop successfully and hatch as three chaetiger larvae from the capsules.

REPOPULATION AND MAINTENANCE

Under natural conditions at Whitstable *P. ciliata* thus has an annual life cycle, the breeding population of each year being formed entirely from the larvae which settled the previous summer. Population density measurements made from the monthly samples gave a mean annual density of 6177 worms/m^2. The ratio of the sexes was one male to 1·17 females. Taking into consideration the bimodal nature of the population, and that the early breeding group have a lower fecundity, it can easily be calculated that the total numbers of larvae released into the plankton is in the order of 3×10^6/m^2. If we suppose

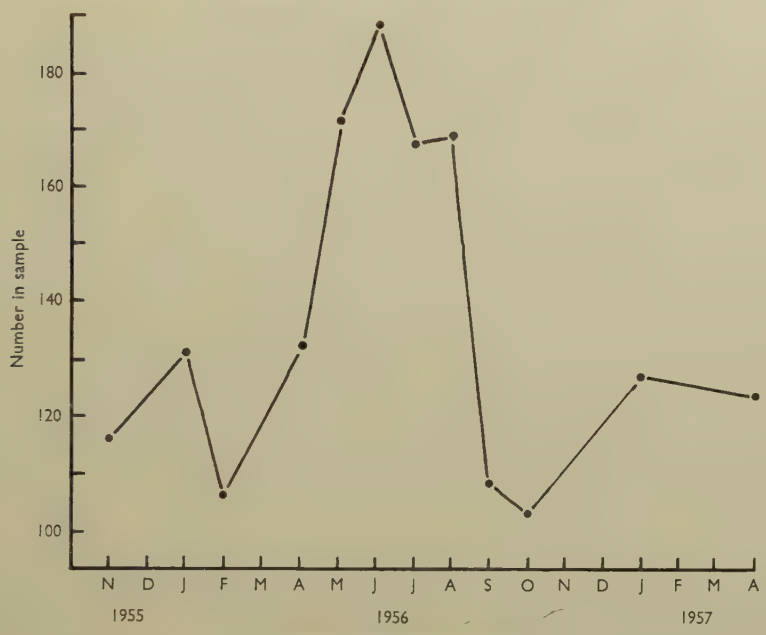

Fig. 8. Annual variation in population density of *P. ciliata*.

that the numbers of *P. ciliata* remain fairly constant from year to year, it follows that there must be an over-all mortality of 99·7 % of the larvae to maintain the balance. The worms reached a maximum density of 190 in the 15 cm^2 sample in June but this fell rapidly in the following months to a winter level of about 120 worms per sample (Fig. 8). This implies that 37 % of all larvae that settle are lost during metamorphosis and the immediate post-larval period. It is not possible to assign values to the contributory causes of predation, dispersion and failure to metamorphose which make up the total loss of the larvae. A suitable substratum appears to be of extreme importance at metamorphosis. Whitelegge (1890) observed that as the larvae approach

metamorphosis they become very sensitive to disturbances such as water currents which cause them to cling to solid surfaces by means of a sticky mucous secretion. Larvae that settle on a surface covered in fine silt are rapidly smothered by the particles sticking to them and perish. This last point should be of interest to those to whom *Polydora* is known mainly as an oyster pest. Oysters growing in still water where silt can accumulate on the shell, are less likely to suffer the heavy infestations of the worm that sometimes affect those grown in areas with a good tidal scour.

DISCUSSION

What little information exists on the time of breeding of *Polydora ciliata* round our coasts indicates that temperature plays an important part in initiating the release of eggs and sperm. At Whitstable in 1956 the temperature of the sea began to rise in February and the larger proportion of the population attained maturity and spawned in March to early April. Wilson (1928) recorded maximum egg production at Plymouth in March, whilst at St Andrews, the larvae first appear in the plankton in July (McIntosh, 1915). This would coincide with egg laying in late May. Allowing for some slight delay in warming of the sea water as we pass from south to north, this suggests that it is rise in temperature that promotes breeding in those worms indigenous to our coasts. Corlett (1948) also connected variations in settlement with variations in temperature. Hannerz (1956) mentions that the population of *P. ciliata* in the Gullmar Fjord also has two spawning periods, the first larvae being found in the plankton in early May to June, and a second group appearing from the middle of September to December. Unfortunately, no figures are available for the fecundity of the adults that produce these larvae.

The difference in fecundity of the two fractions of the population of *P. ciliata* at Whitstable, added to the fact that they restore themselves to approximately the same proportions after the fluctuations of metamorphosis are over, suggests that the events recorded here are not a freak occurrence. *P. ciliata* is a cosmopolitan species, being recorded as burrowing in a wide variety of materials between the latitudes 80° N. and 60° S. It is inevitable that within this range, adaptation to regional environmental conditions must occur. The early breeding worms of the Whitsable population resemble in their fecundity the worms described by Wilson at Plymouth, whilst the larger fraction of the population, which spawns in March to April, has a fecundity similar to the worms from the coast of Sweden.

Newell (1954) considers that the marine fauna of Whitstable is derived from two main sources, namely, animals migrating in a northerly direction up the English Channel and those migrating southwards from the North Sea. At Whitstable, there is perhaps an overlap of populations of *P. ciliata* from an

Atlantic and a North Sea centre of distribution, which differ in their fecundity and in the precise nature of the temperature stimulus required to promote the onset of breeding.

I should like to record my thanks to Professor J. E. Smith, F.R.S., for much useful advice and discussion during the course of this work which was carried out whilst holding a D.S.I.R. postgraduate studentship.

SUMMARY

The germ cells of *Polydora ciliata* arise from cells lying just inside the ventral epithelium and later migrate into the gonads. They are released into the coelom when they are 30 μ in diameter and are transported posteriorly to the region of maturation. The eggs and sperm are shed through the modified nephridia of the region of maturation. The eggs are laid into a string of capsules apparently secreted by the nephridia and attached to the wall of the tube. The larvae take about a week to hatch and are released into the plankton at the three chaetiger stage. They spend some 6 weeks in the plankton before metamorphosis, and have grown to 17 or 18 segments.

Monthly samples of the worms living in the London clay at Whitstable in 1956 showed that the population was bimodal with respect to size and the two groups of adults gave rise to two larval settlements in late March and May. The two groups of juvenile worms that developed from these larvae retained their identities through the summer and the following winter. There is no apparent morphological character by which the adults of each type may be recognized, the time of spawning and the lower fecundity of the early breeding worms being the only distinguishing features. It is suggested that these groups are the consequence of an overlap of two populations of *P. ciliata* from different centres of distribution, each being adapted in its breeding requirements to a different part of the temperature range.

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THE BOTTOM FAUNA OF THE ENGLISH CHANNEL

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The Plymouth Laboratory

(Text-figs. 1–15)

The collections described here form a survey of the bottom fauna along the whole length of the south coast of England. Further collections are being made in mid-Channel and near the French coast, and it is hoped to publish the results at a later date. Partly because of the absence of rich fishing grounds, the bottom fauna of the English Channel has been somewhat neglected in the past, and previous information has been mainly confined to the more western areas, in the neighbourhood of the marine stations at Plymouth and Roscoff. Although the fauna of the Channel is quantitatively poor when compared with the fishing grounds of the North Sea, it provides an interesting study of distribution trends since there are considerable variations both in temperature and in other hydrographic conditions between the wide western end, parts of which were dominated by a *Sagitta elegans* plankton community in the 1920's, and the more land-locked areas of the eastern half, characterized by a *Sagitta setosa* community. In recent years evidence has accumulated that benthos species may usefully supplement plankton species as a perhaps more permanent record of hydrographic conditions. Thus Blacker (1957) has shown that certain species may be used as indicators of either Arctic or Atlantic water in Arctic regions, the correlation being apparently with temperature, while in the North Sea Ursin (1960) has suggested that factors other than temperature may be responsible for the local distributions shown by some echinoderms. In the English Channel both temperature and certain unexplained hydrographic factors appear to play a part in the distribution of different species, and this paper is an attempt to establish the main trends of distribution in the Channel and to correlate them with the hydrography of the area.

I am indebted to Lt.-Cdr. C. A. Hoodless, R.N.R., and the officers and crew of R.V. 'Sarsia' for their skill and patience in working the anchor-dredge, sometimes under difficult conditions. Preliminary sorting of the catches on board ship was only made possible by the generous and able assistance of Dr P. R. O. Barnett, my wife, Mrs M. L. Holme, and Messrs G. R. Forster, E. Harada, A. D. Mattacola, D. J. Scarrett and G. M. Spooner. I am also indebted to Mr E. Ford for allowing me to use his notes on an unpublished grab survey of Great West Bay in 1923, and to Mr L. Birkett for

information on the fauna of the central North Sea, Mr A. D. McIntyre for information on the Scottish area, and Dr J. A. Allen for the Clyde area.

The following have been of assistance in the identification of certain groups: Dr J. A. Allen (*Nucula*), Mrs Eve Southward (*Arenicola*), Mr G. M. Spooner (certain Crustacea), and Dr A. C. Stephen (*Maxmulleria*).

I would like to thank Dr P. R. O. Barnett, Mr L. Birkett, Mr A. D. McIntyre and Dr A. J. Southward for helpful criticism of the manuscript.

Dr Barnett has taken Vacuum-Grab samples at each station for a survey of the microfauna of the sediments, the results of which he proposes to publish at a later date.

METHODS

Collections were made from R.V. 'Sarsia', using a modified anchor-dredge (Fig. 1), which was slightly larger and much more robust than the original model (Forster, 1953). It did not dig as deeply as previous models, and tended to drag for a short distance along the bottom rather than to anchor the ship. It represents a compromise between ordinary dredges, which scarcely dig in at all on sandy grounds, and the original anchor-dredge which digs in

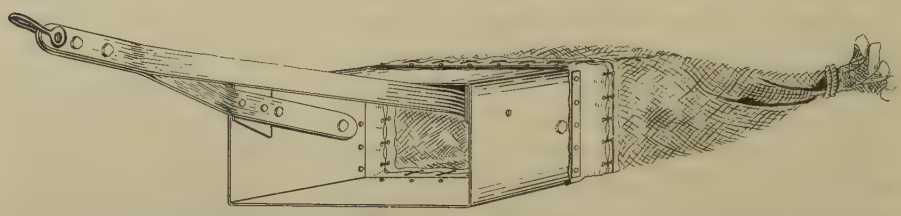

Fig. 1. The modified anchor-dredge. The steel frame forming the mouth of the dredge is 2 ft. (61 cm) wide, 1 ft (30·5 cm) high, and 16 in. (40·7 cm) deep. Overall length of dredge without net is 4 ft (122 cm), and weight *ca* 70 kg. The wishbone arms are set at an angle of 18° to the long axis of the frame, and swivel on the hindmost bolts. They can be locked in position by the set screw in front of this bolt. Reinforcing strips attached to the arms prevent it cutting into the edge of the frame. Drawn by Mr G. A. Battin.

very deeply but which may be unsuitable for use from a large vessel owing to the very considerable strain on the warp when it becomes anchored in the deposit. The new model was designed by Mr Forster to work either way up, the wishbone towing arm swivelling accordingly, but in shallow water it was found to dig more effectively with the arm locked to one side. If lowered right side up while there was some way on the ship, it almost invariably landed on its correct side. In deeper water (over 80–100 m) there may be some difficulty in landing it right way up, and the arm can then be unlocked so that it will dig whichever way it lands.

The new dredge has been worked at over 200 stations on all types of ground, without being damaged, although the net had frequently to be mended or replaced. The net was of No. 2 Courlene of ½ in. (12·7 mm) bar, the lower end being open and tied with a cod-end knot. For work in deeper water a stramin

lining is recommended, but was not used in this survey as a reasonably large sample of deposit was obtained at most stations. In shallow water and in calm weather washing-out of the sample while the dredge is being hauled is not excessive. Only a single haul was taken at most stations. Positions were obtained by Decca Navigator, and depths, from the echo-sounder, were corrected for tidal height based on the nearest port for which tidal data are available.

Treatment of the samples aimed at providing the fullest *qualitative* evaluation of the fauna. Samples of deposit were often very large (100 l. or more), so that it was not always possible to sieve the whole sample. Where the deposit was sufficiently fine it was passed through a sieve with square openings 2·2 mm across, failing this sieves of 5 mm or 11·5 mm were employed. Often only a portion was sieved, and the remainder was then hosed down on deck, conspicuous animals and dead shells being picked out.

Although there are considerable variations in the samples at each station, due both to the volume of deposit brought up, and to its subsequent treatment on deck, the numbers of individuals of each species have been noted as a rough guide to their density on the bottom.

Dead shells were also recorded, on a semi-quantitative basis, and such records are of value in confirming the 'absence' of certain species in particular areas.

VALIDITY OF THE SAMPLES

The anchor-dredge rather than the grab was used in this survey for two reasons: first because it was at first believed that much of the area was rocky or stony and therefore unsuitable for the grab, and secondly because the density of species is in general low in the Channel, necessitating large samples for adequate qualitative sampling. The samples correspond roughly to $\frac{1}{2}$–1 m^2 excavated to a depth of 15 cm, but considerable variations in area and depth sampled clearly exist. The samples appear to be adequate for collecting representative members of the burrowing fauna at each station, but are insufficient for obtaining larger members, mainly of the epifauna, which are known, from trawl catches, to occur on the grounds.

This paper is concerned with the main trends of distribution in the Channel, and for this purpose it was desirable to sample a range of sediment grades in each area, and also to sample at a variety of depths. This ideal could not be fully realised as no two sediments are identical, and in certain areas sediments of a particular grade were absent. Thus coarse deposits are poorly represented in the western half of Great West Bay, and fine deposits are lacking in the tide-scoured area south of the Isle of Wight.

By definition, sampling cannot establish the absence of a species from a particular area. Where the density of a species is very low, it may cease to form a breeding population, and so rank as an 'exotic' perhaps transported in the larval stage from a denser, breeding population elsewhere. Thus we may

be justified in considering a species *normally sufficiently dense to occur in the dredge samples* to be absent, for practical purposes, when it is not taken in a series of dredge-hauls on apparently suitable deposits.

CONDITIONS IN THE CHANNEL

Some of the hydrographic and geological features of the Channel have been summarized by Crisp & Southward (1958), and additional information on water movements in the mouth of the Channel is given by Cooper (1960*a*, *b*). In general, the floor of the Channel slopes gently both along and across its main east–west axis. In the western entrance depths of about 110 m occur in mid-channel, decreasing progressively to a maximum of 50 m in mid-channel in the Straits of Dover. From mid-channel shorewards depths decrease to about 35 m close to the south Cornish and Devon coasts, west of Start Point, sloping more abruptly up to the beach. Consequently the area inside say 20 m is small in extent and often too close to the shore to be sampled. Across Great West Bay and farther east, however, the 35 m contour often lies 10 or more miles from shore. In selecting stations for this survey an attempt was made to sample a range between 10 and 50 m depth in each area. Consequently stations off the Cornish coast lie mainly within a mile or two of the shore, while farther east they are more widely scattered, usually at a greater distance from the coast.

It is now known (King, 1954) that in many parts of the Channel there is only a thin veneer of sediment overlying the rock formations. A 1 m coring tube obtained rock samples from most parts of the Channel, except Great West Bay, and off Plymouth the depth of sediment is only 15–30 cm (Holme, 1953), less in places, with rocky outcrops in some areas. Sediment depths less than about 15 cm would restrict the occurrence of deeper-burrowing species, particularly species like *Lutraria* and *Upogebia*. In most areas, however, a representative burrowing fauna has been taken, indicating perhaps the occurrence of deeper pockets of sediment on grounds where the average depth of sediment is small.

Except possibly in late summer, there is a residual flow of water from west to east up the Channel and into the North Sea. Practically all the water in the Channel (apart from that provided by precipitation) is therefore derived from various water masses lying in different areas and depths off the mouth of the Channel. In passing up-Channel changes in the properties of these waters occur, the most apparent being that of temperature. At the mouth of the Channel surface temperatures fluctuate from about 10° C in February to 16° C in August (Lumby, 1935), while in the Straits of Dover the range is from 6½° to 16° C.

In mid-channel a tongue of east-going water, travelling more rapidly than in coastal areas where eddying may occur, carries the thermal properties of

waters in the western Channel right up through the Straits of Dover. Thus in August the mid-channel water off Dungeness is about $\frac{3}{4}$° C cooler than inshore, while in February it is $\frac{3}{4}$° C warmer.

Data on bottom temperatures are less complete, but the main features are shown in Fig. 2. In winter, complete vertical mixing occurs, so that the surface and bottom temperatures for February are identical. In summer a thermocline is set up in the western half of the Channel, but in the east, because of stronger tidal streams and shallower water, the vertical temperature gradient is small (Matthews, 1911; Dietrich, 1950). It follows that there

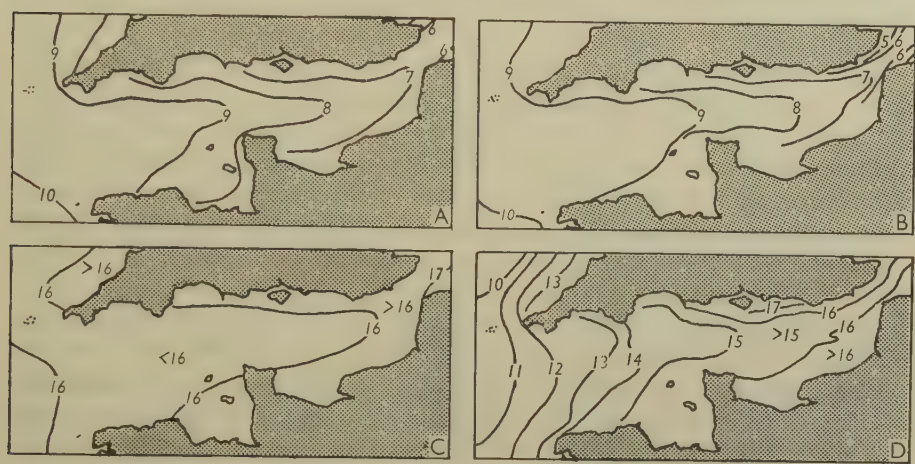

Fig. 2. Isotherms (° C) at surface and bottom for February (A, B) and August (C, D). From Deutschen Seewarte Hamburg (1927).

is a considerable east–west temperature gradient on the bottom in summer, whereas on the surface temperature variations are little more than a degree. Close inshore the existence of eddies and counter-currents may cause local variations in temperature not revealed on the charts, and it seems possible that thermoclines may become temporarily established inshore in the eastern Channel in warm weather.

Surface salinity shows a slight decrease up-Channel (Lumby, 1935). Patches of lower salinity occur near the mouths of rivers, but, apart from these, salinities are not appreciably below 34 ‰ anywhere in the Channel in February. Salinity therefore appears unlikely to be a factor controlling distribution in open Channel waters.

Exposure to Atlantic swell from the west naturally decreases up-Channel, but the shallower water and increased tidal currents east of Portland probably produce a more disturbed bottom in these areas. These effects not only prevent a thermocline from becoming established but also hinder the settlement of silt and clay, which remain in suspension, so making the water more

turbid. There is a progressive increase in turbidity eastward up-Channel, probably accelerated on passing into the eastern basin. Large quantities of silt in suspension may affect the filtering rates and growth of certain lamellibranchs (p. 434) and this factor may prove critical for certain species.

Other factors which change from west to east are summarized by Crisp & Southward (1958), but for the offshore bottom fauna the temperature régime differs from that experienced by the shore fauna, notably in the absence of subzero temperatures and frosts. For this, and for other reasons, too close a comparison should not be drawn between the distribution of the offshore fauna described here and that of the shore fauna (mainly upper and mid-tidal) described by Crisp & Southward.

Apart from the more-or-less fixed pattern of conditions described above, there are long-term changes which may affect the fauna. Southward (1960) has shown a slight increase in water temperatures off Plymouth (at E 1) during recent years, and a more decided increase has been shown by Cooper (1960*b*) from observations made at the Seven Stones Light Vessel. Figures given in the charts of the Deutschen Seewarte Hamburg (1927) and Lumby (1935) should probably be increased by $\frac{1}{2}$–1° C to give present-day values. These temperature changes no doubt follow the amelioration of climate in the northern hemisphere during the present century, and may be expected to allow a northward extension of the range of many species.

Changes in the distribution of water-masses off Plymouth, shown by the replacement in about 1931 of a *Sagitta elegans* plankton community by one characterized by *S. setosa* (Russell, 1935) are well known, and the present position is reviewed by Southward (1961). Earlier grab surveys by Mr E. Ford off Plymouth and in Great West Bay in 1922–23, made when the *S. elegans* community occurred off Plymouth, are valuable for comparison with the present-day bottom fauna under *S. setosa* conditions.

BOTTOM-DEPOSITS AND FAUNA

Pratje (1950) gives a chart showing the general disposition of sediments in the Channel. Much of the bed of the Channel is covered by gravels or sands, and deposits composed largely of mud are very localized. In Great West Bay and Rye Bay in particular there is an abundance of fine quartz sand, but elsewhere other materials form a considerable proportion of the deposits. For example, flints and chalk are a feature of the deposits in the more eastern parts of the Channel, while off much of Devon and Cornwall the Devonian slates of the coastline contribute shaly materials to the inshore deposits. These finally break down to silt and clay, and so do not contribute towards the accumulation of sandy deposits. Calcareous materials are present in nearly all deposits (see Allen, 1899, for analyses of calcium carbonate in sediments off Plymouth), and often accumulate to form characteristic shell-gravels formed mainly of

lamellibranch valves. There are also coralline gravels formed from the remains of the alga *Lithothamnion calcareum* off the Cornish coast.

In few places in the Channel do high population densities approaching those in the North Sea occur. The fauna at the western end of the Channel is distinguished more by the number of species it contains than for their density, but equally dense total populations, comprising fewer species, occur at the eastern end, particularly in Rye Bay.

Although this survey is restricted to inshore areas (Fig. 3), there is some evidence that these contain the more productive grounds. Collections taken farther offshore have usually proved disappointing, with the exception perhaps of the Eddystone region (depth *ca.* 65 m) which appears to be richer in individuals and species than the inshore grounds off Plymouth.

Apart from the Plymouth and Roscoff neighbourhoods, our knowledge of the Channel fauna is very incomplete. There is no general account of the bottom fauna off the Cornish coast west of Looe, nor of the eastern Channel beyond the Isle of Wight. The fauna of Great West Bay is described by Holme (1950), and that of the Dorset coast by Spooner and Holme (1961).

Brief description of areas sampled

MOUNT'S BAY. Much of the bottom on the west side of the Lizard peninsula appears to be rocky, making dredging difficult and unprofitable. Most of the Bay is fully exposed to the south and west, but Penzance Bay is more sheltered and has deposits of gravel and stones, also of muddy sand, supporting a varied fauna. The fauna would probably be rather rich if more sheltered from wave action.

FALMOUTH BAY and the eastern side of the Lizard are sheltered from the west, and the fauna is relatively rich in numbers of species and of individuals. Deposits are varied, and include *Lithothamnion* gravels in parts of Falmouth Bay.

ST AUSTELL BAY is well sheltered from the west and south-west. Present-day deposits appear to be largely derived from washings from the St Austell china clay workings, and include kaolin, quartz sand, and mica flakes. *Lithothamnion* gravels also occur, this being the farthest east that they were found in the survey. Isolated pieces of dead *Lithothamnion* are taken up-Channel, however; on Admiralty charts they are often recorded as 'coral'. Fine muddy sands predominate in the Bay, with a rich and varied burrowing fauna.

PLYMOUTH AREA. Eastward from St Austell Bay the coastline becomes more exposed to the south-west, although such exposure is less than in Mount's Bay. The bottom is patchy with rock, gravel or shale in many places, patches of fine sand occurring in Whitsand and Bigbury Bays. Dense beds of *Ophiothrix fragilis* occur here and elsewhere along the south Cornish coast. There is a noticeable decline in species and individuals compared with the two previous areas.

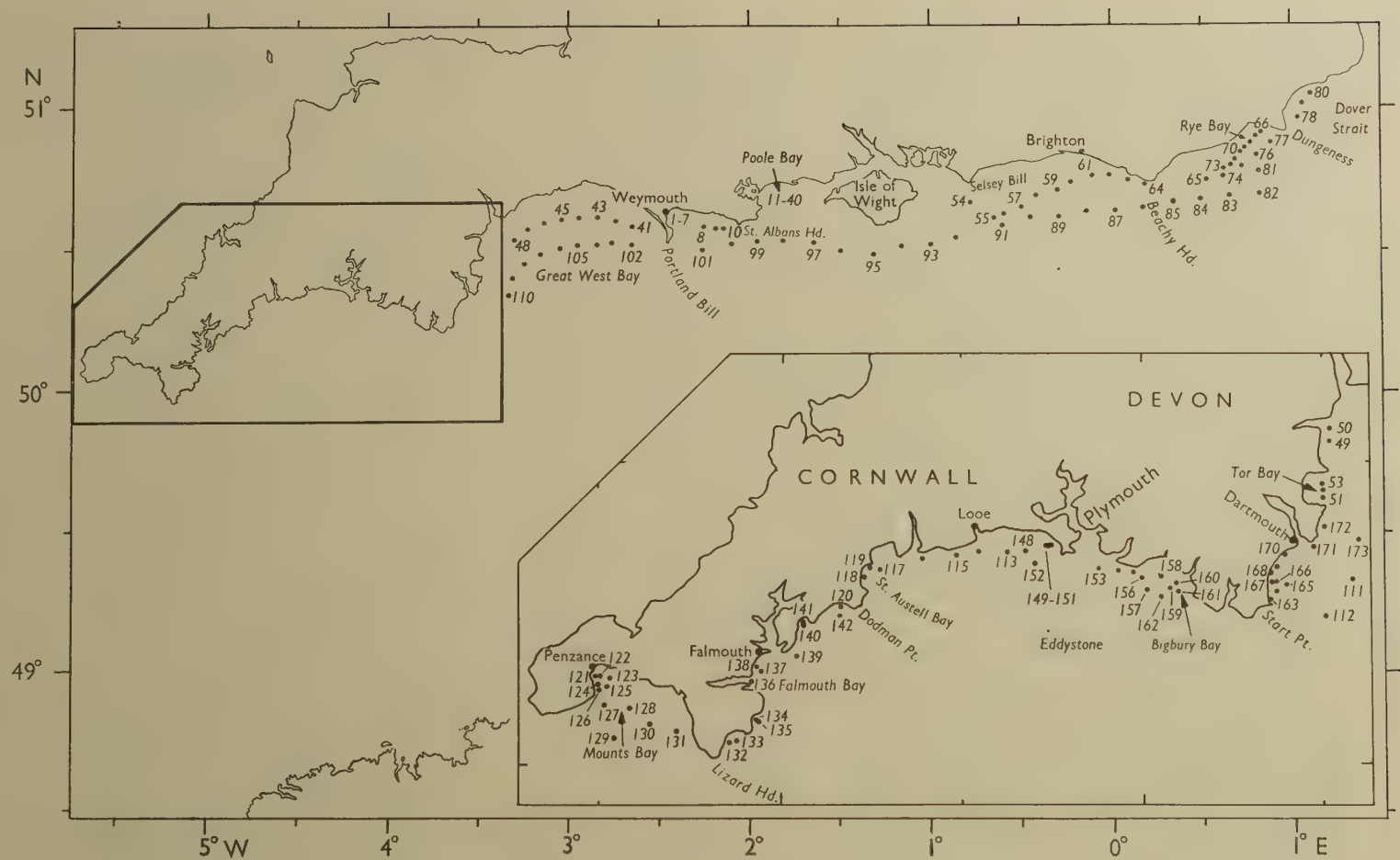

Fig. 3. Chart showing station positions. Where stations lie in sequence along a line, intermediate station numbers are omitted

GREAT WEST BAY. Apart from deposits of gravel in Start Bay and towards Portland the bottom is of fine sand (derived from Permo-triassic sandstones) mingled with a varying proportion of mud. Both tidal streams and wave-action are much less severe than in the Plymouth area (Kyle, 1903), so that muddy deposits are extensive, particularly in and around Tor Bay. Dense beds of certain species occur, among them the *Spisula solida* bed in Start Bay (now less dense than previously), also beds of *Lutraria lutraria*, *Turritella communis* and *Callianassa subterranea* (Holme, 1950).

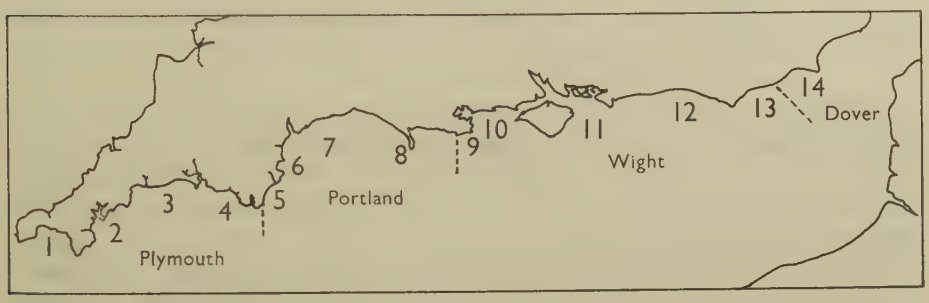

Fig. 4. Chart showing positions of the fourteen areas, each of ten stations, into which the Channel was divided. Meteorological Office sea areas are also shown.

WEYMOUTH BAY. This small bay is well sheltered by Portland Bill and supports quite a rich fauna in mixed deposits of sand, mud, or gravel (Spooner & Holme, 1961), but farther east, off Lulworth, the bottom is of rock or gravel, exposed to greater disturbance by waves and tides, and the fauna is rather poor.

POOLE BAY is relatively sheltered, with deposits of mud, gravel or stones, with clean sand in one part (Spooner & Holme, 1961). The mud tends to be black and anaerobic, a condition rare in offshore deposits in the Channel. The Bay favours certain species, notably *Nucula* spp., but certain sand burrowers such as *Echinocardium* were noticeably absent from the samples. *Crepidula fornicata* is very common in this area.

ISLE OF WIGHT. South of the Isle of Wight the bottom is 'rough' and swept by strong tidal streams. The dredge brought up mainly stones and lumps of chalk, with a meagre attached fauna.

SELSEY BILL TO BEACHY HEAD. The bottom is rather stony with some mud, but with patches of clean sand in places. The fauna is poor.

RYE BAY has a rich fauna on a bottom mainly of muddy sand. A number of species common in the western Channel were absent, but such species as *Nucula turgida*, *Tellina fabula*, *Abra alba* and *Natica alderi* were common. Soft mud, in which the gephyrean *Maxmulleria lankesteri* was common, occurred both west of Dungeness and in black mud off Hythe to the eastward of this point, where three stations were worked.

DISTRIBUTION OF THE FAUNA

Distributions in the Channel in the area surveyed may be classified thus:

(1) Species generally distributed in the Channel.
(2) Species confined to the western parts of the Channel.
  (*a*) 'Western' species mostly restricted at present to the south Cornish coast.
  (*b*) 'Cornubian' species, at the northern end of their range, which penetrate into the Channel from its western end.
  (*c*) 'West Channel' species most of which are widely distributed in European waters, but which disappear abruptly on entering the eastern half of the Channel.
(3) Species distributed around the central region of the Channel.
(4) Northern species, most common in the eastern half of the Channel.

Besides these there are a number of special distributions, including those of shallow-water species, the phenomenon of 'submergence', and the peculiar distribution of *Abra prismatica*.

Not all the species taken in the survey can be placed in one of the above categories, owing to scarcity of records. It is therefore not possible to discuss in detail the distribution of some species known to have a restricted distribution in British waters, such as *Marthasterias glacialis*, *Ophiocomina nigra*, and certain other echinoderms. Dead shell records contribute much to our knowledge of mollusc distributions, so that the distribution pattern for these is more clearly defined than for other phyla.

SPECIES GENERALLY DISTRIBUTED IN THE CHANNEL

This is naturally the largest group, the species of which apparently occur wherever the grade of deposit and other bottom conditions are suitable. The area to the south of the Isle of Wight, being stony, is unsuitable for the majority of burrowing species, but otherwise they are fairly widely distributed. It is noticeable, however, that some are very common in Poole Bay, while others are virtually absent from this area (Fig. 5).

The majority of these species are widely distributed around the British Isles and in European waters:

Nucula hanleyi	Distribution outside Britain uncertain
Nucula turgida	Sweden–Med.[1]
Chlamys opercularis	S. Norway–Med.[1] (var.), etc.
Pecten maximus	S. Norway–Med.[1] (? var.)
Laevicardium crassum	Norway–Med.[1] (var.)
Cardium echinatum	Iceland, N. Norway–Med.[1] (var.), etc.
Lutraria angustior	Distribution outside Britain uncertain
Gari fervensis	Iceland, N. Norway–Med.[1] etc.
Abra alba	S. Norway–Med.[1]

Tellina fabula	S. Norway–Med.
Phaxas pellucidus	S. Norway–Med.
Ensis ensis	S. Norway–Med.[1]
Corbula gibba	Norway–Med.[1] etc.
Nassarius reticulatus	S. Norway–Med.[1]
Natica alderi	Norway–Med.[1]
Ophiura texturata	Iceland, S. Norway–Med. etc.
O. albida	Iceland, N. Norway–Med. etc.
Echinocardium cordatum	S. Norway–Med.

[1] Species recorded by Bucquoy *et al.* (1882–98) from the Gulf of Lions, on the Mediterranean coast of France.

There are also three species which here approach the northern end of their range: *Diplodonta rotundata*, *Tellina donacina* and *Acrocnida brachiata* (see Table 4).

Diplodonta and *Tellina* both occur in south and south-west England (Forbes & Hanley, 1853). It is doubtful if *Diplodonta* occurs in the Clyde (Scottish Marine Biological Association, 1961) and it is not recorded from Orkney (Rendall, 1956) nor off the east coast of Scotland by McIntyre (1958). Mr L. Birkett informs me that it occurs living, but very rarely, on the Dogger Bank. It was not recorded in the latter area by Davis (1923, 1925).

Tellina donacina extends a little farther north, being recorded from Orkney, but not off the east coast of Scotland. It was recorded from the central North Sea by Davis (1925), but not by Birkett in recent years.

Acrocnida brachiata occurred regularly in samples throughout the length of the Channel. It sometimes occurs in muddy sand together with *Amphiura filiformis* but on the whole inhabits less muddy grounds. Ursin (1960) quotes its northern limit as the Swedish west coast, and it seems significant that although not recorded by Davis (1923, 1925) nor mentioned by Stephen (1934) from the North Sea it has more recently been taken at quite a number of stations by Ursin (1960), McIntyre (1958) and Birkett. Ursin considers that the local distribution of this species in the North Sea may be partly related to sea temperatures, and it seems possible that a northward extension of its range has occurred in recent years.

SPECIES CONFINED TO THE WESTERN PARTS OF THE CHANNEL

Western species

Several species were only recorded alive west of Plymouth. A few of these appear to be near their northern limits, and these are described in the next section. The majority, however, are widely distributed north and south of Britain, so that some factor other than temperature may be responsible for their restricted distribution in the Channel. Of these species three or four

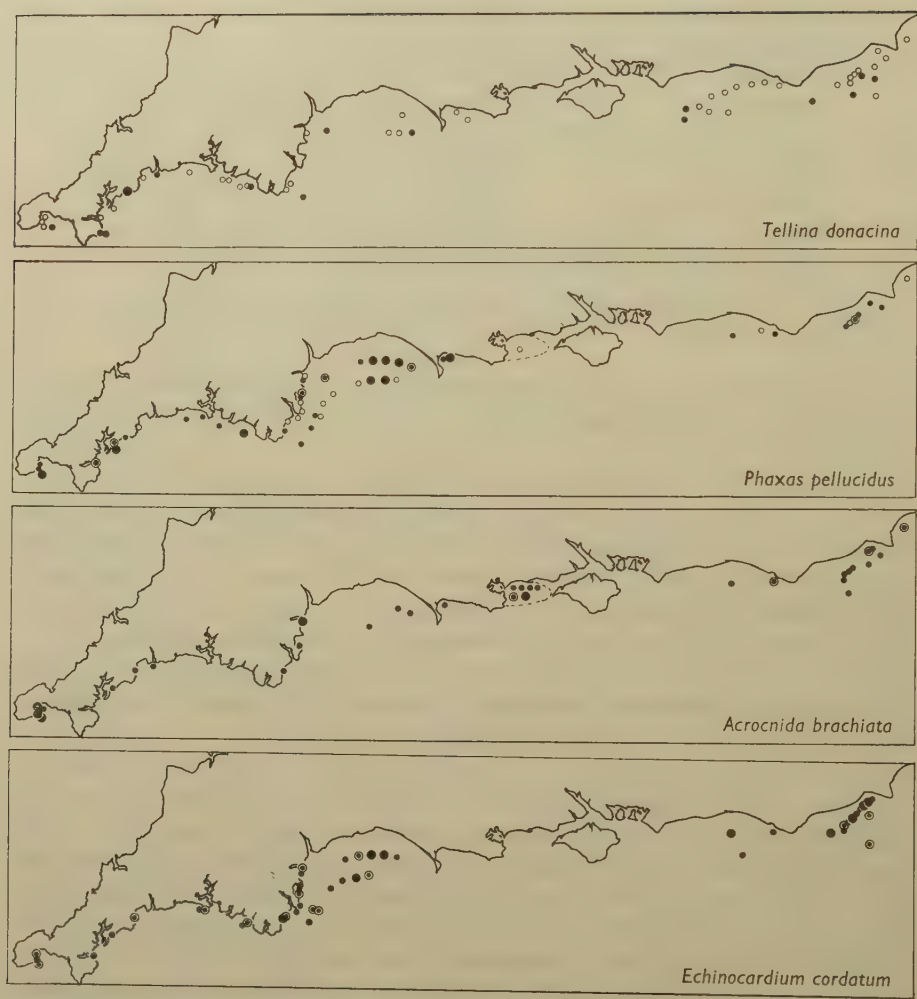

Fig. 5. Occurrence of certain species generally distributed in the Channel, on the basis of the anchor-dredge survey. Positions of stations in Poole Bay are not shown, the area being treated as a whole. ○ dead shells only; (usually an indication that the species is present, but in low density). •, 1–4 living per station; ⊙, 5–10 living; ●, > 10 living.

have a sufficiently consistent distribution in both the Channel and North Sea to rank as 'indicators' of perhaps 'western', but not necessarily *Sagitta elegans*, waters. The indicator species have the following common characteristics.

(i) They are generally distributed on the Atlantic coasts of the British Isles (e.g. Nichols, 1900).

(ii) Small numbers are found alive today off the south Cornish coast, from about Looe, westward.

(iii) The molluscs are represented, as dead shells only, to the eastward no farther than the south-west corner of Great West Bay. Apart from one or two records of single valves south of Rye Bay, there are no records for the rest of the Channel area covered in the survey.

(iv) They were quite common off Plymouth in 1922–23 (Ford, 1923), some occurring in the Sound, but are today absent or very rare offshore (Holme, 1953); while living specimens were recorded in Great West Bay neither by Ford (unpublished data) in 1923 nor by the present author in 1948 (Holme, 1950).

(v) Those present in the North Sea are more or less confined to the north-west area off the east coast of Scotland (Stephen, 1933), characterized by *Sagitta elegans* and *S. serratodentata* communities (information on plankton communities and the hydrography of the North Sea is summarized by Ursin (1960)).

It will be seen from Table 1 and Figs. 6 and 7 that the following species fall into this category.

Myrtea spinifera, inhabiting muddy sand, is perhaps the best 'indicator' species. It is widespread, but not particularly common along the west coasts of Britain and Ireland, and I have found it to be a characteristic species in Dingle Bay in west Ireland and in Quiberon Bay in south Brittany. *Gouldia* (=*Gafrarium*) *minima* is a southern form which does not appear to occur, at least in appreciable numbers, in the North Sea; but *Tellina pygmaea* shows a similar distribution to *Myrtea*. *Thracia villosiuscula* is a quite distinct species, although sometimes confused with *T. phaseolina*. Records from the North Sea and other areas are therefore rather uncertain, but in the Channel its distribution is similar to that of the other indicators. *Gari tellinella* is a little less restricted in distribution, odd valves occasionally being taken at stations up-Channel. Similarly, in the North Sea there is a single record (living) as far south as East Anglia. This species still occurs off Plymouth, but in reduced numbers.

Cochlodesma praetenue is another species with a predominantly western distribution, but the data on this species are insufficient. Off Plymouth it occurs mainly in deeper water near the Eddystone. *Ophiocomina nigra* also has a western distribution in the Channel, and in the North Sea it occurs mainly in the north-west area, but also in the Kattegat and Øresund (Ursin, 1960). Other species apparently showing a western distribution in the Channel, but for which data are inadequate include:

Gari depressa
G. costulata
Chaetopterus variopedatus
Marthasterias glacialis
Echinocardium pennatifidium
E. flavescens
Holothuria forskali

TABLE 1. DISTRIBUTION OF WESTERN AND RELATED SPECIES

(For references see Appendix, p. 460. Symbols for habitat are descriptions of sediments as on p. 456.)

	Geographical distribution	Eastern limit in Channel		Density per 10 m² off Plymouth (Holme, 1953)		Great West Bay (living)						
		Living	Dead	1922–23	1950	1923, 1948	North Sea	Orkney	Bay of S. Malo	Habitat	Feeding method
Myrtea spinifera	S. Norway–Med. etc.	St Austell Bay (4° 45′ W.)	Great West Bay (3° 20·5′ W.)	4·2	0	0	N.W. area	—	—	m.s.	S.
Gouldia minima	Britain–Med.* etc.	Falmouth Bay (5° 03′ W.) (also St Austell Bay)	Great West Bay (3° 29′ W.)	11·8	0	0	—	Common	1, living	gr.	S.
Tellina pygmaea	N. Norway–Med.	Off Looe (4° 22′ W.) (also Plymouth Sound)	Bigbury Bay† (3° 59′ W.)	2·6	0	0	N.W. area and off Jutland	Common	—	gr.	D.
Thracia villosiuscula	Iceland–Med.*	Dodman Point (4° 50′ W.)	Bigbury Bay (3° 55·5′ W.)	0·4	0	0	Dogger, living, rare. Moray Firth	Present	—	s.	S.
Gari tellinella	Iceland–Med.	Bigbury Bay (3° 59′ W.)	Occasional to near Beachy Head (0° 01′ W.)	4·8	1·4	0	N.W. area 1 stn. off E. Anglia	Common	—	gr.	S.
Chlamys tigerina	N. Norway–Britain	—	Great West Bay (3° 18′ W.)	0	0	0	?	Common	—	'rough'	S.
Cochlodesma praetenue	Iceland–S.W. Europe (Med.?)	Great West Bay (3° 29′ W.)	No records farther east	0	0‡	0	E. coast Scotland Dogger, rare	Common	—	s.	S.
Dentalium entalis	Iceland, Spitzbergen–Med.(?)	Mount's Bay (5° 30′ W.)	E. of St Austell Bay (4° 37′ W.)	0	0	0	Northern half	Common offshore	—	s.	C. (Foraminifera)
Ophiocomina nigra	S. Norway–Med. etc.	Whitsand Bay (4° 16′ W.) (also Plymouth Sound)	X	Records insufficient		0	E. coast Scotland, N.E. coast England. Kattegat and Øresund. Dogger	X	X	'rough'	C.

* Recorded by Bucquoy *et al.* (1882–98) from the Gulf of Lions.

† Single valves at Stations 82 and 83, south of Rye Bay (0° 49′, 0° 40′ E.).

‡ Recorded at other stations, near the Eddystone, in 1950.

X, not applicable, i.e. records apply to molluscs only; S, suspension-feeder; D, deposit-feeder; C, carnivore.

The more typical Western species are in bold type.

Chlamys tigerina was represented only by dead shells, although it is taken alive off Plymouth, usually perhaps in deeper water. It is a northern species, here near its southern limits, so may be restricted in the Channel by temperature.

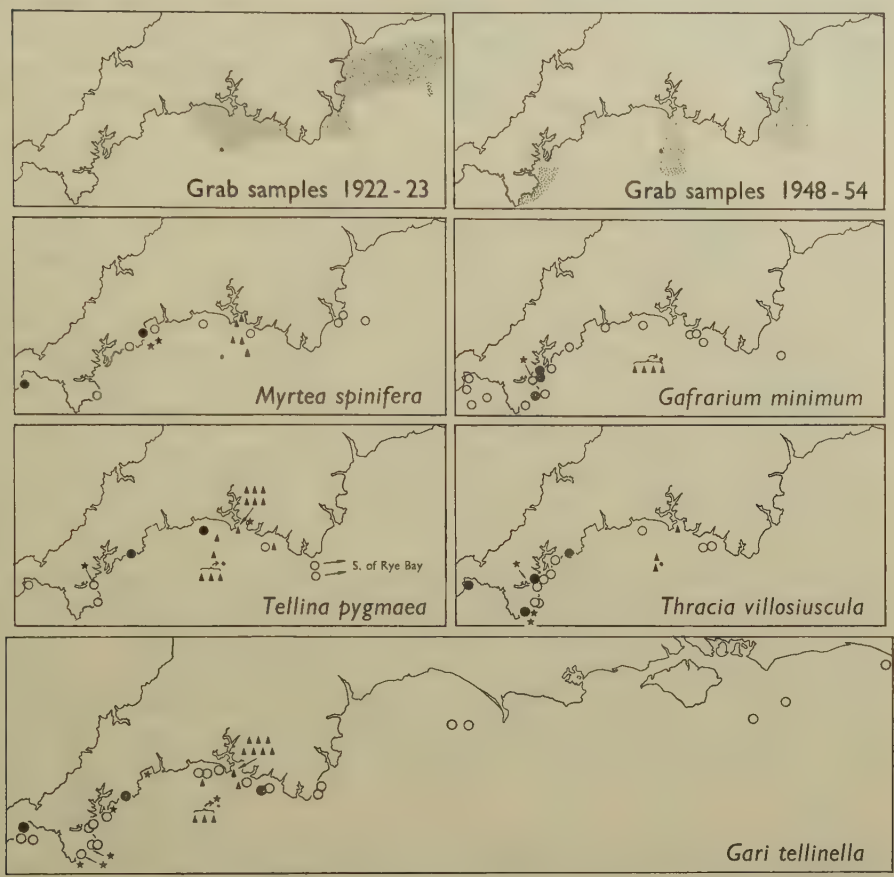

Fig. 6. Distribution of typical Western species. The two upper charts show areas covered by samples other than in the dredge survey (see p. 455). ○, dead shells only; ●, living specimens. ★, other records, 1948–54; ▲, Ford's records in 1922–23. There are no records from parts of the Channel not shown, apart from two dead shell records of *Tellina pygmaea* in the eastern Channel. *Gafrarium minimum* is a synonym for *Gouldia minima*.

The distribution of *Dentalium entalis* (Fig. 10) differs from that of the other species. It was taken living in Mount's Bay at 48 m, and dead-shell records occur to St Austell Bay. I have also taken it living at several positions in mid-Channel south of Land's End (depth *ca.* 120 m), where it is not uncommon. Off Plymouth it was common on the Eddystone grounds at the end of the last century (Allen, 1899), but is now absent from this area. Farther west it is a characteristic species in deeper water in the Celtic Sea (Le Danois, 1948), and

in the North Sea it occurs over most of the deeper northern half (Stephen, 1933); McIntyre (1958) records it in only 30–40 m off the east coast of Scotland, however. Its distribution in the Channel and North Sea is essentially similar, therefore, as it occurs mainly in deeper water but also in shallower depths in the west or north-western areas. Although doubtfully recorded from the Mediterranean, it is possibly near its southern limits in British waters. Kristensen (1959) suggests that it is absent from the southern North

Fig. 7. Distribution of certain Western species in the North Sea, based on Stephen (1933). Occurrence of species in each International Fisheries square is shown. The records include those of Davis (1923, 1925) in the central and southern North Sea, and of Hagmeier (1925) in the German Bight. H, a single record of *Tellina pygmaea* by Hagmeier; ●, *Myrtea spinifera*; ★, *Tellina pygmaea*; ▲, *Gari tellinella*.

Sea because of excessive bottom disturbance there, and this may well be of importance in restricting its distribution. The distribution of *D. entalis* is almost complementary to that of *D. vulgare* which occurs in the central and eastern Channel (Fig. 10).

The absence of most of the Western species from the Bay of S. Malo suggests that penetration up-Channel on the French coast may be similar to that on the English side.

Cornubian species

There are a few species (Table 2) whose northern limits lie in British waters and which penetrate a short distance into the Channel from the west. Unlike the more typical Western species of the previous section these show no evidence of a decline in numbers off Plymouth in recent years. An exception is the southern species *Gouldia minima*, which belongs to the previous section. Although their relative abundance in more western areas (particularly on the shore and in shallow water around Falmouth) may be partly due to the same conditions which also favour Western species, it seems possible that their penetration to the eastward is limited by the lower winter temperatures there.

Around the British Isles these species are mainly restricted to the south-west, being scarce or absent off the Isle of Man, in the Clyde, off Orkney and in the North Sea.

As these species approach the limits of their range they seem to disappear from the shore, but may still be present offshore. This is discussed later, but it may be noted that there appears to be no rigid boundary to the extension of these species, and they may be expected to occur here and there in favourable localities beyond the limits suggested in the table. *Labidoplax* extends considerably farther up-Channel than the other species, its final limit being perhaps at the boundary of the east and west halves of the Channel.

West Channel species

Both the Western and Cornubian species discussed above are relatively uncommon in the areas of the Channel in which they occur, but there are also many species both widely distributed and common in north European waters which are absent from that part of the eastern Channel covered by the survey. They do not gradually decline in numbers on passing up-Channel, indeed many are more common in Great West Bay than farther west, but all disappear abruptly near the boundary between the west and east halves of the Channel, in the region of St Alban's Head. Weymouth Bay provides the most easterly record for many, and apart from *Amphiura filiformis* none was found living in Poole Bay. Dead shell records from the eastern Channel are negligible, and the only species found alive was one *Dosinia exoleta* south of Rye Bay (station 81). The apparent sharpness of this boundary is probably enhanced by three factors. One is related to conditions in Poole Bay which

TABLE 2. DISTRIBUTION OF CORNUBIAN SPECIES

	Geographical distribution	Eastern limits in Channel survey		Occurrence at low-water mark							
		Living	Dead	Roscoff	Bay of S. Malo	Falmouth area	Plymouth area	Isle of Man	Clyde	Orkney	North Sea
Callista chione	Britain–Med.* etc.	—	St Austell Bay (4° 43·5′ W.)	Common	—	Common locally	(Offshore only)	—	—	—	—
Tellina squalida	Britain–Med.* etc.	Gerrans Bay (4° 56′ W.)	Weymouth Bay (2° 25′ W.)	Common	Present	Occasional	V. rare	2 records	Rare	—	—
Solecurtus scopula	Britain–Med.*	—	Bigbury Bay† (3° 59′ W.)	—	—	—	(Offshore only)	Few records	V. rare	V. rare	—
Labidoplax digitata	Britain–Med.	Great West Bay (2° 51′ W.)	X	Present	X	Common locally	Occasional	—	Rare	X	—

* Recorded by Bucquoy *et al.* (1882–98) from the Gulf of Lions.

† Also one valve at Station 81, south of Rye Bay. Other symbols as in Table 1.

appear to be unfavourable to certain species distributed throughout the Channel (p. 406) and which might be similarly unfavourable to West Channel species. The second is the nature of grounds south of the Isle of Wight, which are stony and tide-swept, and the third is related to the sampling techniques.

At stations 1–10 in Weymouth Bay and 11–25 in Poole Bay, dead shell records are not complete as they represent merely a selection of species, other than those found living, without any attempt at a comprehensive list. No species occurring in any numbers could have been missed, but some of the scarcer ones may have been overlooked. Stations 26–40, also in Poole Bay, are

TABLE 3. DISTRIBUTION, HABITAT AND FEEDING HABITS OF WEST CHANNEL SPECIES

	Geographical distribution	Orkney	Central and southern N. Sea	Habitat	Feeding habits
Lucinoma borealis	N. Norway–Med. etc.	Common	At 8 stations (Davis)	s.	S.
Dosinia exoleta	N. Norway–Med.*	Common	At 18 stations (Davis)	gr.	S.
D. lupinus	Iceland, S. Norway–Med.*	Common	Dogger, uncommon (Birkett)	s.	S.
Venus casina	S. Norway–Med.*	Common, local	—	gr.	S.
V. fasciata	S. Norway–Med.*	Common	Common (Birkett)	gr.	S.
V. striatula	N. Norway–Med.*	Common	Common	s.	S.
Arcopagia crassa	S. Norway–Med. (?)	Local	Dogger, v. rare (Birkett)	gr.	Selective D.
Solecurtus chamasolen	S. Norway–Med.* etc.	—	—	m.s.	S.
Thracia phaseolina	Iceland–Med.* etc.	Common	Dogger (Birkett)	s.	S.
Pandora pinna	Scandinavia†–Med. etc.	—	—	s.	S.
Turritella communis	S. Norway–Med.* etc.	Common	Fairly common	m.gr.	S.
Callianassa subterranea	British Isles	X	At 5 stations (Davis)	f.s.	D.?
Upogebia stellata	Sweden–Med. etc.	X	—	m.gr.	S., C.?
Astropecten irregularis	Iceland, S. Norway–Med.	X	Generally distributed	s.	C.
Amphiura filiformis	Iceland, S. Norway–Med.	X	Generally distributed	m.s.	Selective D.
Ophiura affinis	Iceland, N. Norway–Med.(?)	X	Generally distributed	s.	?
Cucumaria elongata	S. Norway–Med.	X	Local, S.W. and E. of Dogger	m.	S.
Leptosynapta inhaerens	S. Norway–Atlantic coast of France	X	At 9 stations (Davis)	m.s.	Non-selective D.

* Recorded by Bucquoy *et al.* (1882–98) from the Gulf of Lions.

† Jeffreys (1862–9) states that this species ranges northward to Spitzbergen. This is incorrect and may be due to confusion with *Pandora glacialis* Leach.

Symbols as in Table 1, p. 310, and for habitat as on p. 456.

still less complete, and do not necessarily include all living specimens, and dead shells are not usually recorded. In all, thirty stations were worked in the small area of Poole Bay, so that positive records are probably fairly complete.

TABLE 4. OCCURRENCE OF WEST CHANNEL SPECIES ALONG THE CHANNEL

Occurrence noted in each of the fourteen areas of the Channel (see Fig. 4). Numbers of stations at which each species was recorded are shown. Records from other stations additional to those representing each area are shown, preceded by a plus sign. D, only dead shells recorded; (D), only one or two shells or valves recorded in all.

In this and succeeding tables additional records for Poole Bay are all included in area 10.

	Mount's Bay			Start Point				St Alban's Head						Rye Bay
Area ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14
West Channel species														
Lucinoma borealis	1	2+1	D	1	D	—	—	1	—	—	—	—	—	(D)
Dosinia exoleta	2	2	D	D	D	—	D	D	D	—	—	—	—	1
D. lupinus	4	3+1	4	3	D	D	3	D	—	—	—	—	—	—
Venus casina	D	1	D	—	—	—	—	D	2	—	—	—	—	—
V. fasciata	1	3	1	D	1	—	D	1	D	—	—	—	(D)	—
V. striatula	D	3+1	D	1	3	7	5	5	—	—	—	—	—	—
Arcopagia crassa	1	2	D	3	—	—	D	—	D	—	—	—	—	—
Solecurtus chamasolen	2	D	3	2	—	4	D	D	—	—	—	—	—	—
Thracia phaseolina	D	2	1	—	1	1	2	D(?)	—	—	—	—	—	—
Pandora pinna	—	1	—	1	1	—	D	1(?)	—	—	—	(D)	—	—
Turritella communis	D	+1	1	D	1	5	4	3	—	(D)	—	—	—	—
Callianassa subterranea	2	—	1	1	1	6	7	3	—	—	—	—	—	—
Upogebia stellata	—	1	2	—	—	—	1	1	1	—	—	—	—	—
Astropecten irregularis	1	3	2	2	—	1	4	1	—	—	—	—	—	—
Amphiura filiformis	3	—	4	3	6	10	7	5	—	2+1	—	—	—	—
Ophiura affinis	—	1	—	1	1	1	1	—	—	—	—	—	—	—
Cucumaria elongata	—	—	—	2	—	1	1	—	—	—	—	—	—	—
Leptosynapta inhaerens	2	—	—	2	—	6	3	2	—	—	—	—	—	—
Southern species, generally distributed in Channel (for comparison)														
Diplodonta rotundata	1	1+1	1	1	1	D	2	1	—	—	1	1	D	2
Tellina donacina	1	2+1	1	1	1	D	1	1	D	—	2	D	3	1
Acrocnida brachiata	4	1	2	—	1	1	2	3	—	5+1	—	2	5	4+1

'Negative records' provided by the absence of both living specimens and dead shells are however less reliable. At more offshore stations (96–101) in the area a complete record was taken. So far as they go these do not dispute the view that the boundary of West Channel species lies in the St Alban's Head region.

Hardly any of the West Channel species appear to be at the limit of their geographical range in the Channel (Table 3), and their distribution (Table 4, Figs. 8, 9) may be contrasted with those of the southern species which occur mainly in the central area of the Channel (p. 419), or with the three southern species shown in the table which are present throughout the length of the Channel.

The local distribution of West Channel species within the western half of

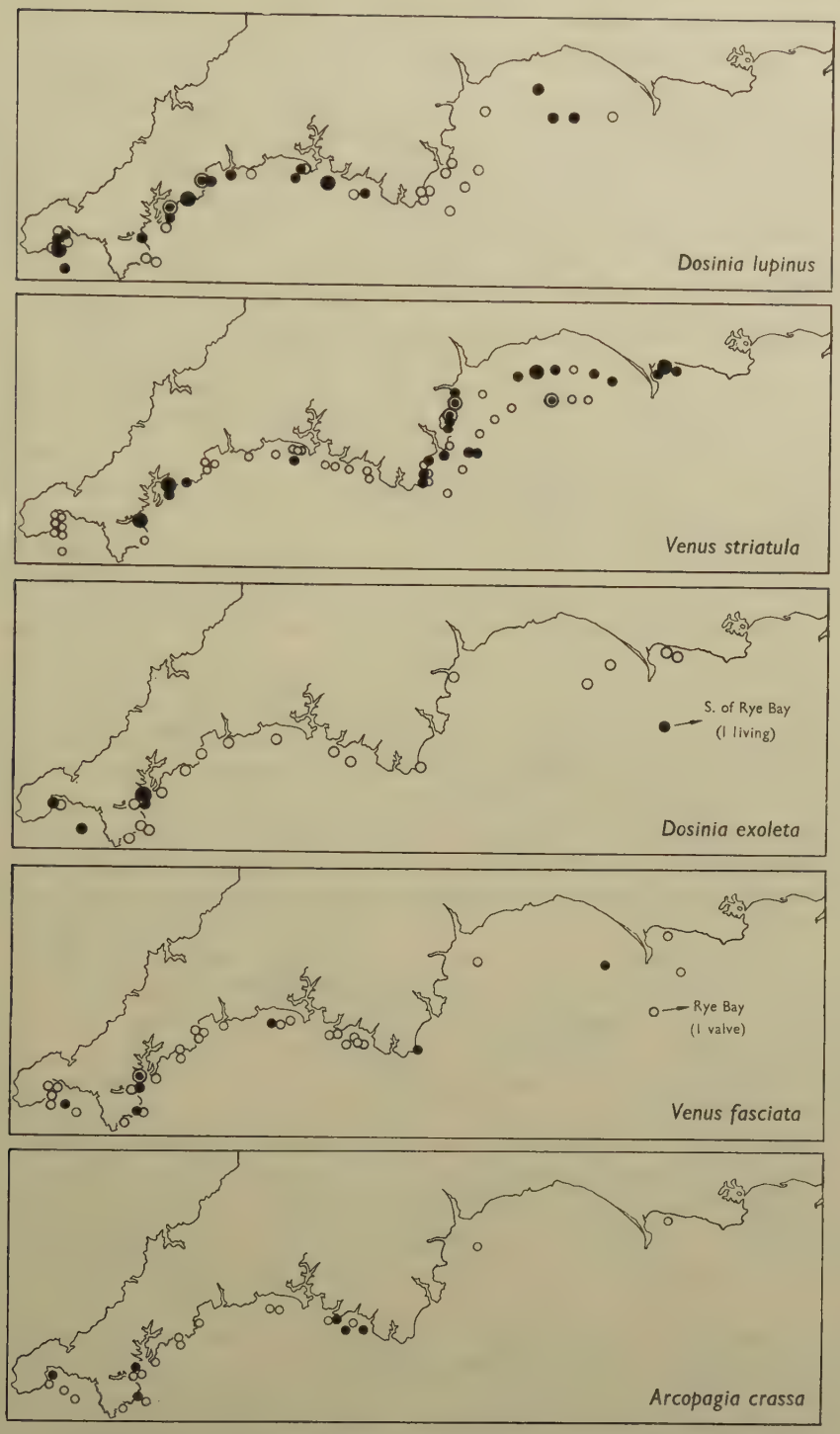

Fig. 8. Distribution of some West Channel lamellibranchs, showing also the effect of soil grade on local distribution. *Dosinia lupinus* and *Venus striatula* inhabit sands, the rest gravels. Symbols as in Fig. 5, p. 408.

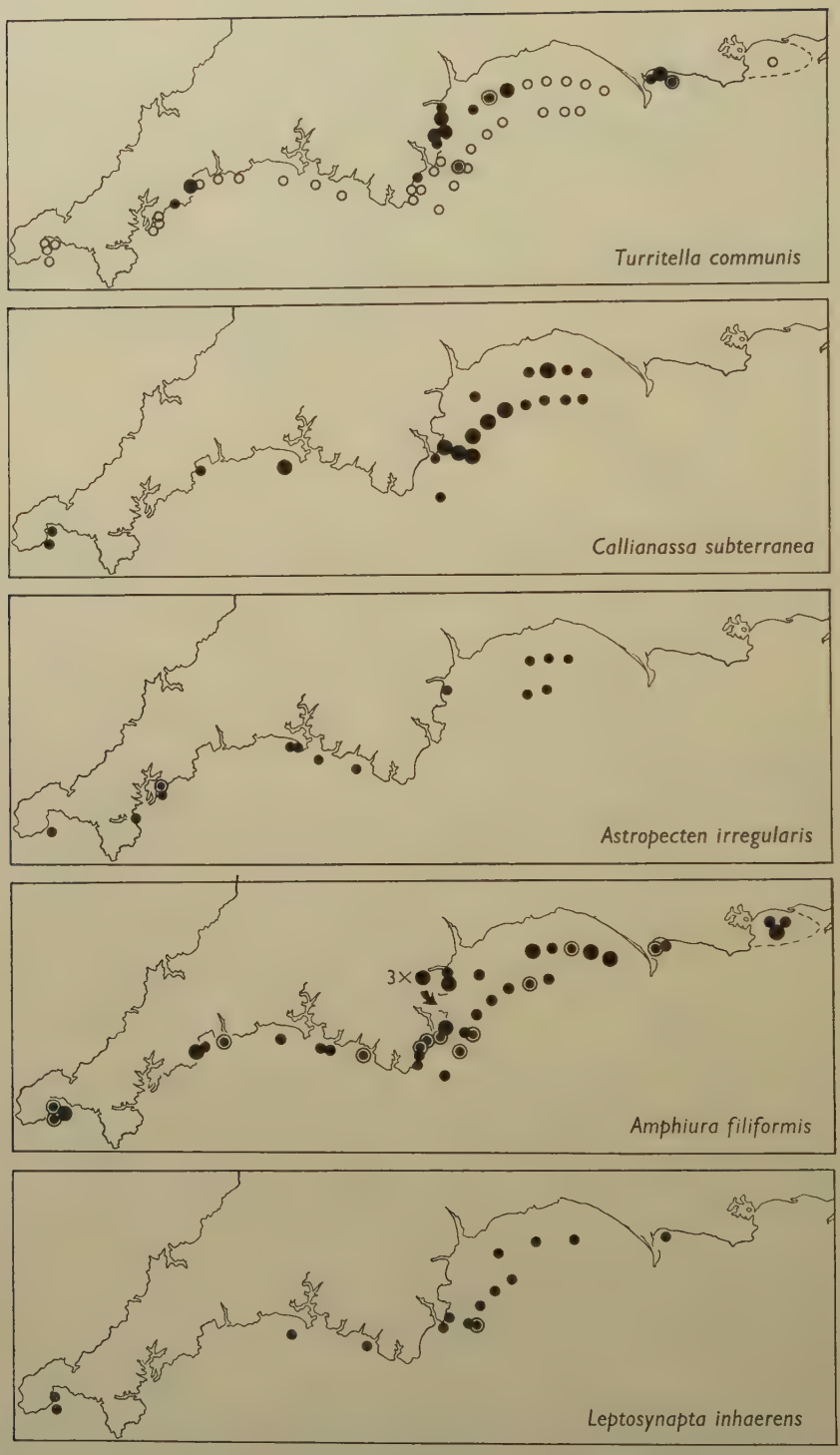

Fig. 9. Distribution of some more West Channel species. Symbols as in Fig. 5, p. 408, except for *Turritella*, where the density scale is: •, 1–10; ⊙, 11–100; ●, > 100.

the Channel appears to be mainly related to soil preferences. There is a close similarity in the distributions on the one hand of *Dosinia exoleta*, *Venus fasciata* and *Arcopagia crassa* inhabiting gravels and on the other of *Dosinia lupinus* and *Venus striatula* inhabiting sands (Fig. 8). Some of the less common species are rather patchy in distribution and some difficulty was experienced in deciding if *Lucinoma borealis*, for example, really belonged to this category. In addition to the species listed, there are a few others which appear to be mainly confined to the western Channel. These include *Glycymeris glycymeris*, *Thyasira flexuosa* and *Lutraria lutraria*.

Feeding habits, so far as they are known, are given in the table. It will be seen that among the lamellibranchs there is a preponderance of suspension feeders, suggesting that some factor such as suspended silt might be responsible for their absence from the eastern Channel. The possible causes of the boundary are discussed later, but it may be remarked at this stage that species occurring in the eastern basin do include a number of suspension feeders, such as *Ensis* spp., *Spisula* spp. and *Venus ovata*.

SPECIES DISTRIBUTED AROUND THE CENTRAL REGIONS OF THE CHANNEL

These reach their maximum abundance between Start Point and Beachy Head. West of Start Point they become rare or are even absent, but some occur between tide marks in the Falmouth area (Fig. 10). To the east they mainly range as far as the dredge survey was carried, and no doubt some occur along the Belgian and Dutch coasts. This group is made up primarily of species near the northern end of their range, but with them are listed certain other species (*Venus ovata*, *Spisula subtruncata*, *Crepidula fornicata*) showing a similar distribution (Tables 5, 6).

The southern forms generally occur in greater abundance off the French coast, particularly in the Channel Islands—S. Malo region, and are therefore referred to here as Sarnian species. Many inhabit rather shallow water, and also occur at low water, particularly on the French coast. As they approach their northern limits there is a tendency for them to occur only offshore and not between tide marks. Some appear to have a bimodal distribution in the Channel: for example *Chlamys varia* and *Venerupis aurea* occur offshore in the central Channel, are rare or absent at Plymouth, and again appear as common species at low water in the Falmouth area. It is curious that offshore records of *Chlamys varia* in the Falmouth area are virtually nil, suggesting an elevation of the depth range inhabited rather than an extension on to the shore from offshore grounds.

Although found more commonly offshore at Plymouth, *Gibbula magus* and *Calyptraea chinensis* tend to a similar bimodal distribution. (The distribution of *G. magus* is discussed by Crisp & Southward, 1958). Both *Dentalium vulgare* and *Nassarius pygmaeus* are virtually restricted to grounds east of

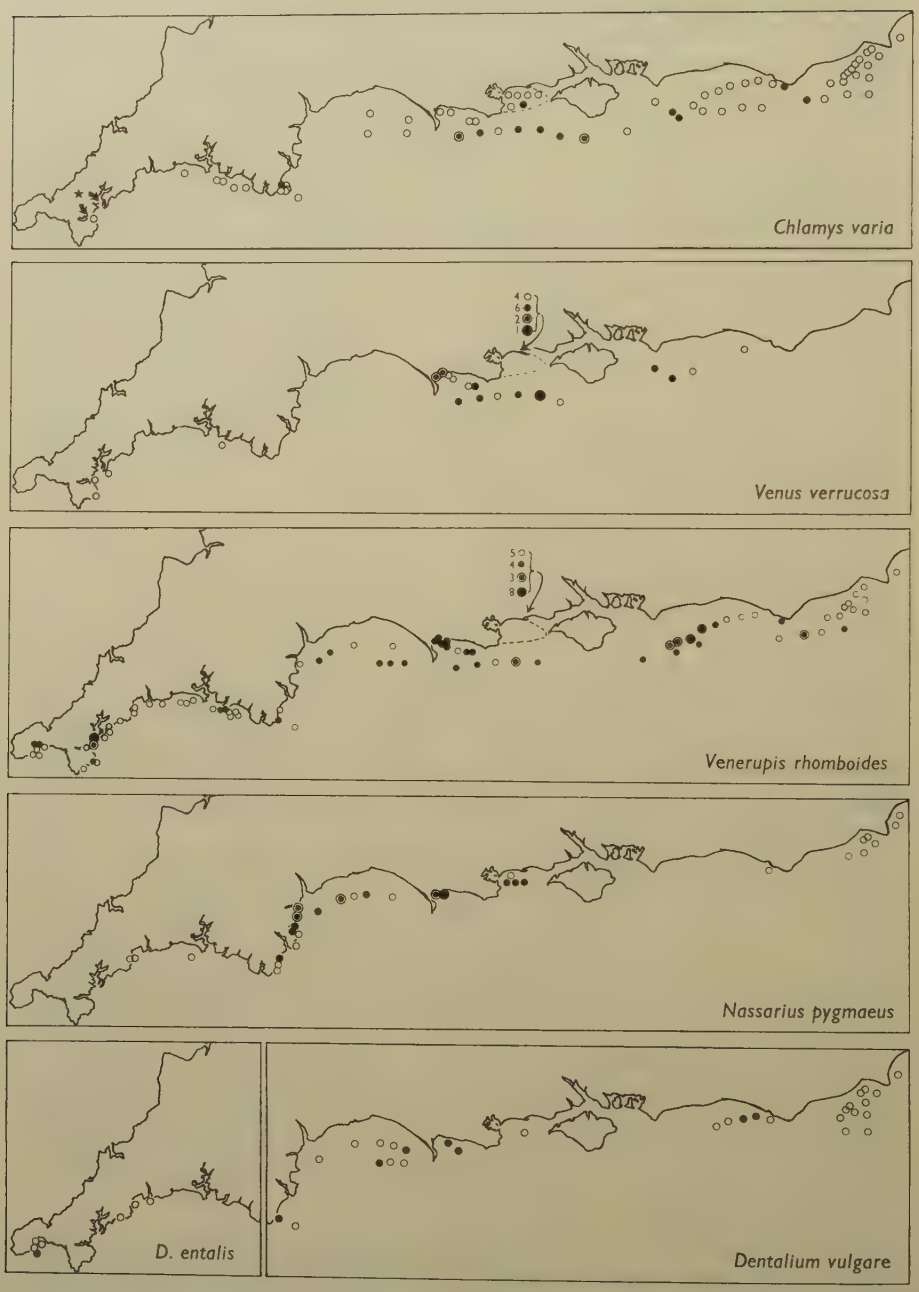

Fig. 10. Distribution of some Sarnian species, also of *Dentalium entalis* as contrasted with *D. vulgare*. Symbols as in Fig. 5, p. 408. ★, shore records of *Chlamys varia*.

TABLE 5. OCCURRENCE OF SPECIES DISTRIBUTED AROUND THE CENTRAL REGIONS OF THE CHANNEL

	Geographical distribution	Roscoff	Bay of S. Malo	Falmouth area	Plymouth area	Clyde	Orkney	North Sea
Nucula nucleus	S. Norway–Med.*	C.†	C.	Probably present	Occ.	C.	C.	N.E. coast Scotland, Dogger, etc.
Chlamys varia	S. Norway–Med.* etc.	Quite C.†	C.†	C†	R.†	Scarce†	C.	At 1 station (Davis)
Venus verrucosa	Britain–Med.* etc.	Quite C.†	Abundant†	? R.†	Occ†.	R.	—	—
V. ovata	Iceland, N. Norway–Med.* etc.	C. offshore†	C. offshore†	? Occ.	Occ.	Fairly C.	C.	Present
Venerupis rhomboides	S. Norway, Faeroes–Med.*	C. offshore†	C.†	C.	C. offshore†	Occ.	C.	—
V. aurea	S. Norway–Med.*	C.†	Abundant†	C.†	—	R.	—	Dead shells, Dogger (Birkett)
Spisula subtruncata	N. Norway–Med.*	Occ.†	Present†	Occ.†	Occ.	C.†	Moderately C. (? on shore)	Dogger, etc. Less common than formerly (Birkett, Ursin)
Pandora albida	Isle of Man–Med.*	Quite C.†	Occ.†	—	R.†	—	—	—
Dentalium vulgare	Britain–Med.* etc.	Occ.†	C.†	—	—	—	—	—
Calliostoma zizyphinum	Norway–Med.*	C.†	C.†	Present†	C.†	C.†	C.†	?
Gibbula magus	Britain–Med.* etc.	C.†	C.†	Quite C.†	R.†	Fairly C.†	C.†	?
Calyptraea chinensis	Britain–Med.* etc.	C.†	C.†	C.†	Quite C.† (occ. on shore)	Common, L. Ryan (? introduced)	—	?
Crepidula fornicata	Introduced	—	—	See Cole† (1952)	R.†	—	—	?
Nassarius pygmaeus	S. Norway–Med.* etc.	R.	Uncommon†	—	V.R.	R.	—	?

* Recorded by Bucquoy *et al.* (1882–98) from the Gulf of Lions.

† Occurs on shore. In nearly all instances the species is also recorded offshore.

C, common; Occ., occasional; R, rare; V.R., very rare.

The more typical Sarnian forms are in bold type.

Start Point, but the majority of other Sarnian species do occur here and there along the coasts west of this headland. *Venerupis rhomboides* is quite common throughout the Channel, but is particularly abundant in the central area, especially in Poole Bay. *Calliostoma zizyphinum*, usually known as a rocky shore form, was taken offshore in the central and eastern areas of the Channel, but was represented only by a single dead-shell record west of Start Point. Crisp & Southward's (1958) data on this species, however, suggest that this species is confined on the shore to the western half of the Channel, both on the French and English coasts. This appears to be another example of an elevation of the depth range in more western areas.

TABLE 6. OCCURRENCE OF CENTRAL CHANNEL SPECIES ALONG THE CHANNEL

Occurrence noted in each of the 14 areas. For symbols see Table 4

	Mount's Bay			Start Point				St Alban's Head					Rye Bay
Area ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14
*Nucula nucleus**	—	—	—	—	—	—	1	2	3	4+4	1	—	1	+1
Chlamys varia	—	D	D	D	1	—	D	D	2	2+1	4	D	2	D
Venus verrucosa	—	D	—	D	—	—	—	2	4	2+8	2	D	—	—
V. ovata	D	D	D	1	1	1	1	2	3	1+4	4	D	D	D
Venerupis rhomboides	2	3	D	2	1	D	3	6	7	4+11	6	2	3	D
V. aurea	—	—†	—	—	—	—	—	2	—	—	—	—	—	—
Spisula subtruncata	D	1	D	D	2	3	3	6	—	2+3	—	D	D	D
Pandora albida	—	—	—	—	—	—	—	1	—	1	—	1	—	1
Dentalium vulgare	—	—	—	—	1	—	1	1	2	D	—	2	D	D
Calliostoma zizyphinum	—	—	—	D	1	D	D	—	3	1	2	D	1	D
Gibbula magus	D	D	D	—	D	—	—	2	1	—	—	—	D	—
Calyptraea chinensis	—	1	—	—	1	D	1	2	—	+1	D	—	—	—
Crepidula fornicata	—	—	—	—	3	1	D	3	3	6+4	4	1	1	—
Nassarius pygmaeus	—	—	D	D	1	3	4	2	—	3	—	D	D	D

* Dead shells not recorded. † Common, living, at low water around Falmouth.

The distribution of *Crepidula fornicata* in the dredge survey accords with what is already known of this North American immigrant. It has long been established in the Solent, and has more recently spread westward, first to Weymouth Bay and then to Great West Bay, where it now occurs in moderate numbers (Orton, 1950). It is still rather rare off Plymouth (Marine Biological Association, 1957), but was introduced independently into the Falmouth area (Cole, 1952).

Two species widely dispersed in European seas are at present mainly confined to the central areas of the Channel: *Venus ovata* and *Spisula subtruncata*. Dead shells occurred throughout the Channel, but live specimens, sometimes in considerable numbers, were taken mainly between Start Point and the Isle of Wight (Fig. 11). Both still occur off Plymouth, where there is evidence (Holme, 1953) of a decline in numbers of *Venus ovata* between

1922–23 and 1950. Comparable data for *Spisula subtruncata* are insufficient. Possibly the more muddy grounds in Great West Bay, Weymouth Bay and Poole Bay are particularly favourable for both these inhabitants of muddy sands, but in *S. subtruncata* there is evidence of a former decline in northern British waters (Elmhirst & Stephen, 1929; Fisher, 1931), and in the North Sea where it is no longer the dominant species on the Dogger Bank (Ursin, 1952; Birkett, 1953). It is possible that the restricted distribution of *Spisula* in the Channel is a reflexion of such a general decline and may represent either a partial withdrawal to a more favourable environment in the central Channel or to an overall decline in density throughout the Channel.

The species in this section are, by definition, not confined to the western half of the Channel. It would seem possible however that certain Sarnian species might be prevented from entering the eastern Channel for the same causes which exclude the typical West Channel species. Such species would be mainly confined, on the English side, to Great West Bay, and possibly occur in smaller numbers farther west. *Callianassa subterranea* (Fig. 9) may possibly be an example of this type of distribution.

NORTHERN SPECIES

One or two northern species (e.g. *Chlamys tigerina*; possibly also *Dentalium entalis*) are restricted to the western parts of the Channel, but two other species *Spisula elliptica* and *Buccinum undatum* are mainly confined to the central and eastern areas. Their distribution in the Channel (Table 7, Fig. 11)

TABLE 7. OCCURRENCE OF SOME NORTHERN SPECIES (ALSO *APHRODITE*) ALONG THE CHANNEL

Occurrence noted in each of the 14 areas. For symbols see Table 4

	Mount's Bay				Start Point				St Alban's Head					Rye Bay
Area ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Spisula elliptica	D	D	D	D	1	D	D	1	4	2+4	2	5	3+1	2
Buccinum undatum	—	—	D	—	1	2	D	2	2	4+1	2	2	3	1
Maxmulleria lankesteri	—	—	—	—	—	—	—	—	—	1	—	—	—	1+2
Aphrodite aculeata	—	—	—	—	—	1	2	2	1	2	1	—	6	4

is not in fact very different from that of many southern species described in the last section, but whereas the latter may be influenced by high summer temperatures, the northern species are perhaps dependent on the lower winter temperatures which occur in the eastern Channel.

The exact geographical range of *Spisula elliptica* is uncertain, owing to possible confusions of identity, but it appears to range at least from Iceland to the British Isles (Madsen, 1949). It may extend down the west coast of

France, but is absent from the Mediterranean. In the dredge survey it was taken living from Start Point eastward, occurring mainly in the eastern half of the Channel. Dead shells only were recorded westward to Mount's Bay. Ford (1923, 1925) describes it as common off Plymouth, but insufficient material is now available to check his records, some of which were in fact of *S. solida* (see Appendix). The late Dr D. Atkins has kindly shown me shells of specimens used for her work on ciliary currents (see Appendix), and some

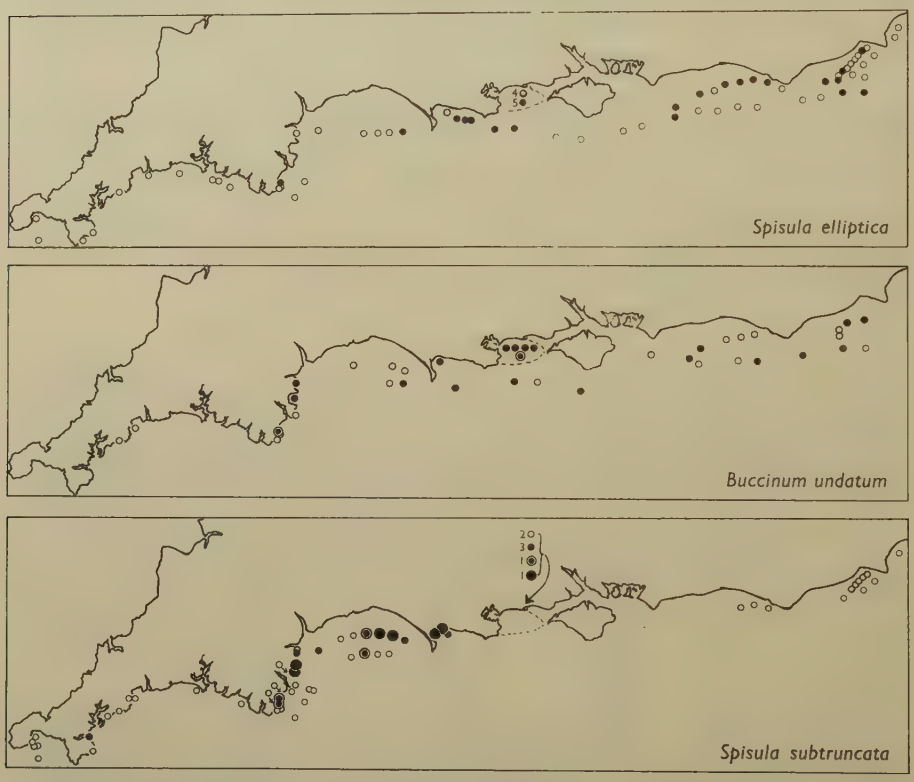

Fig. 11. Distribution of two northern species, and also of *Spisula subtruncata*. Symbols as in Fig. 5, p. 408.

of these have been confirmed as *S. elliptica*. This species was therefore living in the Plymouth area in the 1930's, at least, but its presence here in the 1960's has yet to be established.

According to Jeffreys (1862–69), *Buccinum undatum* occurs from Spitzbergen to La Rochelle. I have recently (1960) taken it alive in Quiberon Bay, so that it still extends into the Bay of Biscay. In the dredge survey it was quite common east of Start Point, but to the west was represented by only two records of dead shells. It is, however, regularly taken in small numbers when

trawling off Plymouth and also at low tide on the shore, particularly in January and February when it comes in to spawn. Offshore it is evidently sparsely distributed, and was not recorded in the photographic survey of the sea-bed off Plymouth made by Vevers (1952). Its absence in the dredge samples illustrates the limitations of the anchor-dredge (or grab) for sampling larger members of the fauna, particularly epifauna species which may be highly gregarious. On the other hand, the regular occurrence of *Buccinum* in dredge-hauls east of Start Point shows that it is very much more common in that area.

The echiuroid *Maxmulleria* (= *Thalassema*) *lankesteri* was taken in soft muddy deposits both in Poole Bay and around Dungeness. There are not very many records of the occurrence of this species (Stephen, 1960), but it appears to be a northern form here at the southern limits of its range. Stephen's record of south Devon as a locality appears to be incorrect, and the present records are probably the most southerly so far.

One other species, *Aphrodite aculeata*, occurred mainly in the eastern Channel, but also in Great West Bay. It has a wide geographical range, from Iceland and Greenland to the Mediterranean. Although not recorded off Plymouth in the dredge survey, it is taken regularly on the trawling grounds. Its occurrence therefore resembles that of *Buccinum*, being far more common in the central and eastern parts than in the west, but unlike that species it is not, apparently, near the limits of its range in this area.

DEPTH DISTRIBUTION

Certain species recorded in the dredge survey are restricted to rather shallow water, probably having a zone of maximum abundance between low-tide mark and *ca.* 10 m depth. The occurrence of such species in the dredge-hauls is sporadic, as it was possible to sample such depths only in a few areas. Additional information from grab and shore collections indicates that a few of these, such as *Callista chione* and *Tellina squalida*, do have a distribution restricted to certain parts of the Channel, but there are others, probably occurring throughout the Channel, which appear to have a patchy or restricted distribution owing to the scarcity of samples at the right depths: these include *Donax vittatus*, *Ensis siliqua* and *Spisula solida*. The majority of species taken in the survey showed little or no evidence of depth restriction, within the depth-range of the samples.

Ursin (1960) discusses the phenomenon of 'submergence' and has shown that certain echinoderms inhabit greater depths in more northern latitudes, while to the south they may extend on to the shore on the Atlantic coasts of France and in south-west England. This is a special example of submergence, not, apparently, previously recorded (cf. Ekman, 1953, for other types of submergence), and Ursin does not consider it to be primarily connected with temperature.

The same trend is shown, to a lesser extent, in certain lamellibranchs living in the Channel. Various species typically occurring offshore at Plymouth are found at low water around Falmouth, the Scilly Isles and on the north Brittany coast. Several of these are near the northern end of their range, and it would appear that in these circumstances they only occur below low-tide mark (Table 8). There also seems to be a tendency for certain species to have an increased tolerance of wave-action in the Channel Islands and on the Brittany coast. For example, *Donax vittatus* occurs on the shore only in certain semi-sheltered areas (particularly Tor Bay) in south-west England, but in south Brittany is common on the fully wave-exposed shores on the west side of the Quiberon peninsula (Prenant, 1932, and my own observations). Similarly, *Solen marginatus* occurs in sheltered creeks and estuaries in south-west England, but in Jersey on wave-exposed beaches. A similar distribution has been noted for *Ensis arcuatus* and *E. ensis* (Holme, 1954). Such differences may be partly due to higher average temperatures in winter in these south-western areas, which enable these species to burrow more actively to avoid wave disturbance; possibly the relative scarcity of frosts and near-zero temperatures is significant.

Another factor which may affect depth distribution is related to the distribution of water masses in the Channel. There is reason to believe that certain species restricted to western areas of the Channel inshore may penetrate far up-Channel in the deeper water offshore. Several examples have been noted of such species occurring at the deeper-water stations (81–83) south of Rye Bay. Isotherms and isohalines tend to lie in chevron formation pointing up-Channel (Fig. 2), and one may suspect that the boundaries of certain species assume the same pattern, since other factors of which the isotherms and isohalines are merely an indicator may be similarly distributed. Hence the occurrence of certain species in deeper water in the eastern Channel than in the west is not related to depth *per se*, but to the distribution of water masses.

NOTE ON *ABRA PRISMATICA*

Ford (1923) described *A. prismatica* as a characteristic species of deeper-water (i.e. over *ca.* 40 m) communities in silty sand off Plymouth, but he also recorded it from Bigbury Bay in 10–20 m. Although quite common in deeper parts of the Channel, this species is by no means confined to these depths, and occurs for example at several stations at about 10 m in Rye Bay. Its distribution in the dredge survey was sporadic, and may be connected with the avoidance of low salinities or high turbidity near the mouths of larger rivers. Wherever it occurred close inshore it was always at some distance from appreciable freshwater inflow. These observations support those of Jones (1956) in Manx waters.

SPECIES ABSENT OR RARE IN THE SURVEY

Many species known to occur off the north Brittany coast do not normally range northward to the English coast. Some occur quite commonly at Roscoff, but are usually doubtfully included in the British fauna. Among burrowing species are:

Divaricella divaricata
Donax variegatus
D. trunculus (not at Roscoff, but occurs in west Brittany)
Mactra glauca
Leptosynapta galliennei
Labidoplax thomsoni (not at Roscoff, but occurs in west Brittany)
Sipunculus nudus (rare in British waters; one at Station 127).

All these species may be found at low-water mark on parts of the Brittany coasts.

Beside these are some other species worthy of note which were absent or rare in the dredge survey.

Pinna fragilis appears to have occurred regularly at one time off the south Cornish coast (Clark, 1906), particularly south of Dodman Point; off Plymouth and at Salcombe (Marine Biological Association, 1957). At present it appears to be extremely rare in the Channel, although several fresh half-grown, empty paired valves have been trawled in 1960 off Plymouth. It is common off Penmarc'h on the south Brittany coast, and apparently ranges northward up the west coast of Britain to Shetland, and southward to the Mediterranean.

Astarte triangularis is so small that it may have passed through the 2·2 mm sieve used in the survey, so that the absence of records may not be significant. It has apparently declined in numbers in the Eddystone shell-gravel in recent years (Plymouth Marine Fauna, 1957), and this, coupled with its predominantly western distribution in British waters (Forbes & Hanley, 1853; Jeffreys, 1862–69) suggests that it may be another Western species. It is distributed from Britain to the Mediterranean.

Pharus legumen has a curious distribution. It is a southern species, ranging from the Mediterranean to the west coast of Britain (Yonge, 1959), burrowing in sand at low tide and in shallow water. Although known from off the *north* coasts of Devon and Cornwall, in Wales and on the Brittany coasts, it is virtually absent from the English Channel coast. It is common on certain beaches near Roscoff (Station Biologique de Roscoff, 1951), but is not listed farther east from the Bay of S. Malo (Dautzenberg & Durouchoux, 1913–14), nor does it appear to occur in the Channel Islands. The few records from the south coast of England (e.g. Forbes & Hanley, 1853) must rank as exotics. I have not seen a single valve on any beach in south Devon or south Cornwall. There is no evidence of any alteration in the range of this species.

TABLE 8. OCCURRENCE OF SPECIES AT LOW-TIDE MARK ON THE SHORE, SHOWING 'SUBMERGENCE'

C, common; Occ., occasional; R, rare; V.R., very rare.

	Geographical distribution	Roscoff	Bay of S. Malo	Scilly Isles	Falmouth area	Plymouth area	Salcombe	Tor Bay
Chlamys varia	S. Norway–Med.* etc.	C.	C.	V.R.	C.	R.	V.R.	—
Dosinia exoleta	N. Norway–Med.*	C.	R.	C.	C.	Occ.	V.R.	—
D. lupinus	Iceland, S. Norway–Med.*	Occ.	—	Occ.	Occ.	R.	R.	V.R. ?
Callista chione	Britain–Med.* etc.	Occ.	—	Occ. ?	Occ.	—	—	—
Venus verrucosa	Britain–Med.* etc.	Occ.	C.	R.	R.	Occ.	V.R.	—
Venerupis aurea	S. Norway–Med.*	C.	C.	Occ.	C.	—	—	—
Tellina squalida	Britain–Med.* etc.	Occ.	Occ.	Occ.	Occ.	—	V.R.	—
Thracia phaseolina	Iceland–Med.* etc.	Occ.	Occ.	—	Occ.	V.R.	R.	—
Pandora albida	Isle of Man–Med.*	Occ.	Occ.	—	—	V.R.	—	V.R. ?

* Recorded by Bucquoy *et al.* (1882–98) from the Gulf of Lions.

TABLE 9. DISTRIBUTION IN CHANNEL OF THREE SELECTED GROUPS

	Lamellibranchia		Gastropoda		Echinodermata	
Areas	No.	%	No.	%	No.	%
A only	13	18·1	—	—	10	38·5
B only	6	8·3	2	14·3	—	—
C only	6	8·3	—	—	—	—
A+B	11	15·3	2	14·3	7	26·9
A+C	3	4·2	—	—	2	7·7
B+C	7	9·7	5	35·7	—	—
A+B+C	26	36·0	5	35·7	7	26·9
Total species	72	99·9	14	100·0	26	100·0
Total species in each area, and percentage of total in Channel						
A	53	73·6	7	50	26	100
B	50	69·4	14	100	14	53·8
C	42	58·3	10	71·4	9	34·6

SOME ASPECTS OF DISTRIBUTION

Table 9 shows the distribution of the three main groups in different areas of the Channel. For this purpose the Channel was divided into three areas, approximately corresponding to the Meteorological Office weather forecasting sea areas (Fig. 4):

(A) *Plymouth*: Mount's Bay to Start Point (45 stations).
(B) *Portland*: Start Point to St Alban's Head (46 stations).
(C) Eastern area, *Wight* plus *Dover*: St Alban's Head to Straits of Dover (76 stations).

In the lamellibranchs there is no great decrease in numbers of species on passing up-Channel. Although several species are restricted to *Plymouth*, the total is maintained in *Portland* by the presence of additional species of the Sarnian group, which are also present in the Eastern area. Among the gastropods, none is restricted to *Plymouth*, and only two species to the western half of the Channel. Sarnian species in areas B and C swell the numbers in these areas, so that the *Plymouth* area is the poorest in numbers of species. The reverse trend is seen in the echinoderms, in which 65·4 % of the total species are restricted to the western half of the Channel, and all the recorded species occur in *Plymouth*. The Sarnian type of distribution is not represented, and there is consequently a considerable fall in numbers of species in the Eastern area. The occurrence of a few species of lamellibranchs and echinoderms in areas A and C but not in B is not considered significant, as it is probably due to sediment requirements.

This analysis indicates the relative suitability of different groups as indicators of conditions in the Channel area. The lamellibranchs are considered the most suitable for the following reasons.

(*a*) They are the most abundant in terms of numbers of individuals and species, and they occur at a higher proportion of stations than any other group.
(*b*) They show a better balance of population trends, as outlined above, than do the gastropods or echinoderms.
(*c*) Additional evidence provided by dead mollusc shells is of value in certain circumstances.

These remarks apply only to the Channel area and to the scale of sampling employed. However, they accord with the views of Stephen (1934) who considered that 'the molluscs should be chosen as the main group for dividing off the natural areas in, at any rate, the North Sea, and that the echinoderms may be used for making further subdivisions, if desired'.

For a quantitative study of total populations the Channel was divided into fourteen areas, from west to east, each containing ten stations (see Fig. 4 and Appendix). A few stations were eliminated to make the areas comparable.

Total numbers of species in each area are shown in Fig. 12; these show the same general trends noted above.

Totals of individuals are shown in Figs. 13 and 14. The lamellibranchs show three distinct peaks, corresponding to areas 2 (Falmouth Bay), 8 (eastern part of Great West Bay and Weymouth Bay) and 14 (Rye Bay), and similar

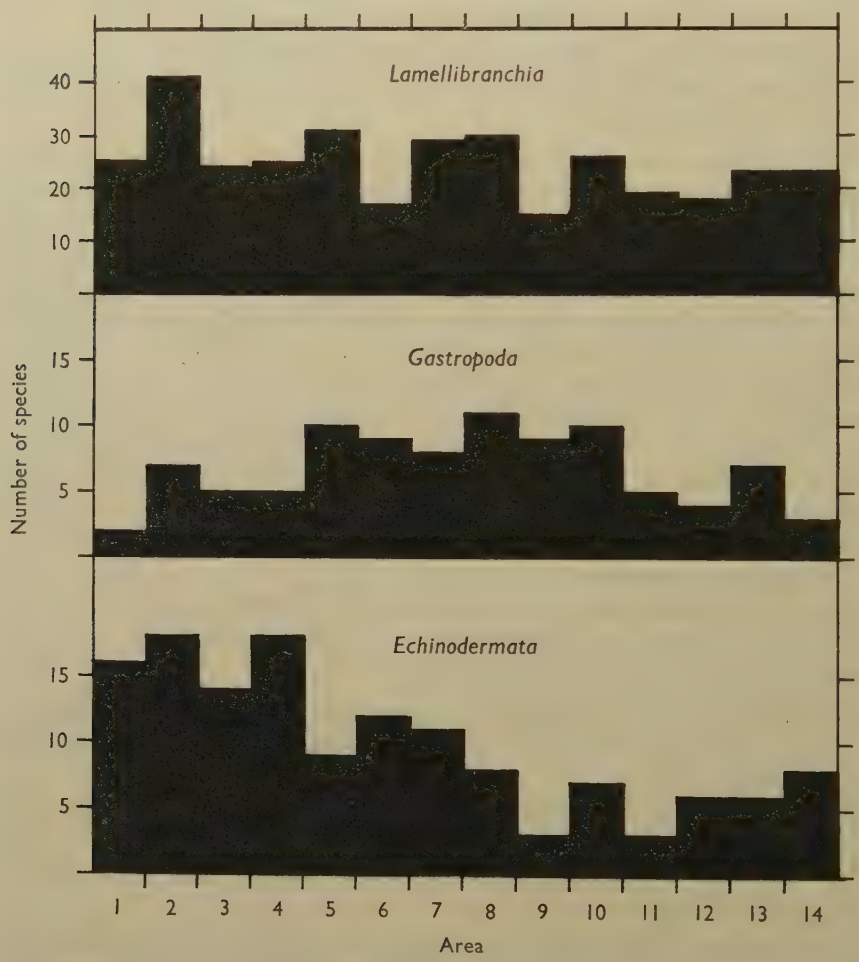

Fig. 12. Numbers of species recorded per ten stations in each area of the Channel. Areas are shown in Fig. 4: the areas run from west to east, area 1 being Mount's Bay.

peaks may be discerned, but not in precisely the same areas, in the gastropods and echinoderms. Areas 3 and 4, lying respectively west and east of Plymouth, are the poorest in numbers of lamellibranchs and are not very rich in gastropods and echinoderms.

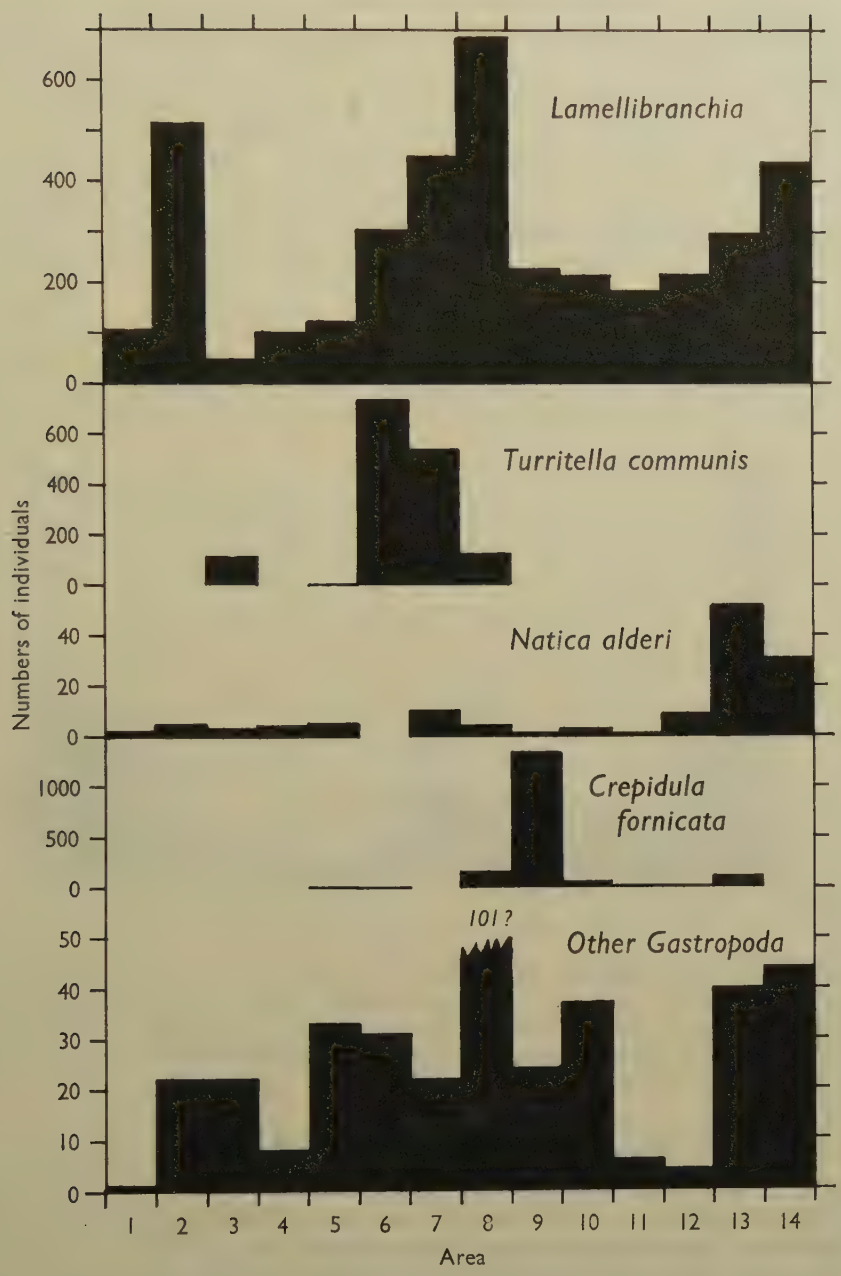

Fig. 13. Numbers of individuals of lamellibranchs and gastropods recorded in each area.

While it is true that variations in the efficiency of sampling and sorting would tend to affect all groups equally, so producing corresponding variations from area to area, it is considered that the histograms do in fact afford a measure of the relative densities of populations in each area. There is no evidence of any over-all fall in density of individuals, a measure of the standing-crop, on passing up-Channel.

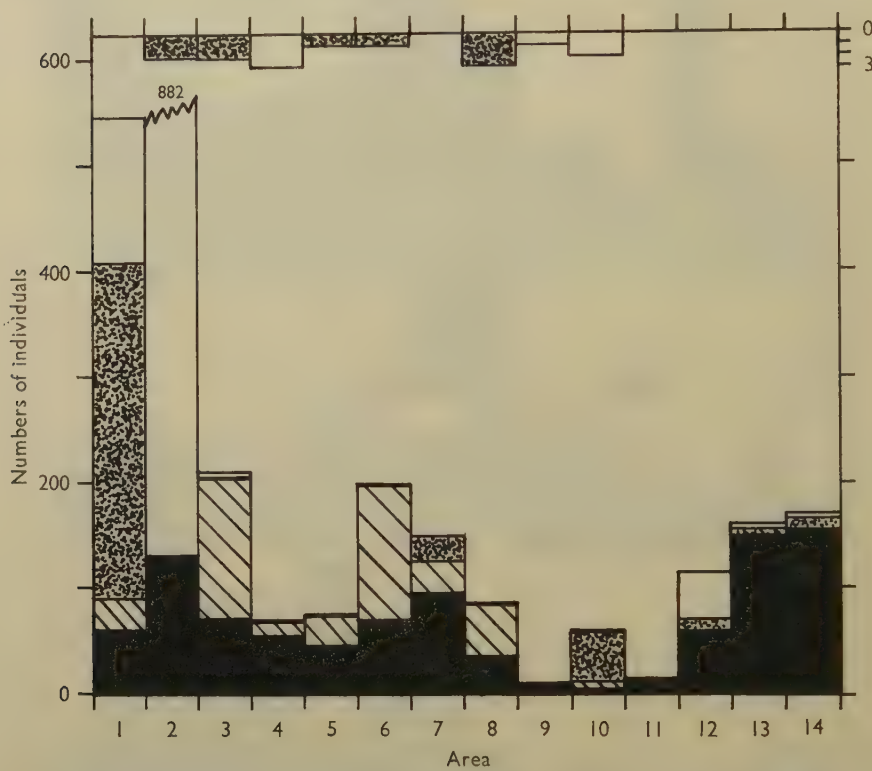

Fig. 14. Numbers of individuals of echinoderms recorded in each area. ▧, *Amphiura filiformis*; ▤, *Acrocnida brachiata*; □, *Ophiothrix fragilis*; ■, others. At the top are shown numbers of *Acrocnida* and *Ophiothrix* on a × 10 scale in areas where they are not common enough to appear on the lower histogram. Most of the *Acrocnida* in area 1 were recorded at one station, similarly with the *Ophiothrix* in area 2.

EVIDENCE FROM DEAD SHELLS

There is little information available on the period for which dead shells may persist on the bottom, and this must in any case be greatly influenced by local conditions. Lamellibranch valves still united by the ligament are clearly not long dead, but the rate of break-up of the valves will be dependent mainly on whether they remain buried in the sediment or lie on the surface, soon to be broken up by current or wave disturbance. There are clearly great differences

in the fragility of shells; some delicate forms such as *Mactra corallina*, *Thyasira* or *Thracia* must persist for a much shorter time than thick-shelled species such as *Nucula*, *Arca* or *Glycymeris*. Birkett[1] has estimated the survival time of intact valves of *Mactra* on the Dogger Bank, from which he has evolved a 'half-life' formula giving the rate of break-up of species of different shell thickness. Apart from radiocarbon or similar dating methods, however, there is no method of telling the age of the individual shells found in a deposit. Since there is little or no *net* deposition of sediment in the Channel the depth in the deposit at which a shell is found probably has little bearing on its age.

While it is fairly certain that dead shells can remain buried *in situ* almost indefinitely, if transported along the bottom the chances of being broken up are greatly increased so that one might expect a limit to the distance which they can be transported, based on the thickness, size and strength of the shell.

Evidence from dead shell remains in the Channel is fairly consistent with the present and presumed past distribution of the mollusc fauna. The virtual absence of dead shell records of West Channel species east of St Alban's Head, both living and dead shells of the commoner species reaching about the same limits, indicates at least no general transport of sediments in an easterly direction. It does not rule out, however, the possibility of movement in a westerly direction. Since the chances are that at least a proportion of the shells would remain *in situ* in the sediment in which they lived, it is probably safe to say that the boundary of West Channel species has never been farther east than it is at present.

With the Western species there is evidence from dead shells that some occurred in the past in the south-west corner of Great West Bay. It seems unlikely that these would have been transported any appreciable distance without breaking up, and since the evidence from West Channel species is against a movement of shells in an easterly direction, it seems fairly certain that at some time in the past 'western' influences reached as far as this part of Great West Bay.

For the other types of distribution the limits are less definite, and a few dead shells are often recorded beyond the areas in which living specimens were taken. In most instances this probably results from a gradual falling-off in population density and not from any change in distribution. Since dead shells often outnumber living specimens by a hundred or even a thousand to one it may be expected that they would be found over a wider area than the records of living specimens.

Apart from a few fossil shells, no evidence of past faunas significantly different from those of today has emerged.

[1] I.C.E.S. report, 1960 meeting.

DISCUSSION

THE ACTION OF LIMITING FACTORS ON BENTHIC ORGANISMS

Temperature is naturally an important factor controlling geographical distribution. At the northern end of their range species may be dependent on high summer temperatures to enable spawning to take place (Orton, 1920), and sufficiently low winter temperatures may be required by species near the southern limits of their range. Crisp & Southward (1958) consider that a certain level of temperature through the year may be important for some intertidal species, but to what extent this is ever a critical limiting factor is questionable. Abnormally low winter temperatures, above freezing-point, are sometimes lethal (e.g. Ursin, 1960), and it is possible that certain (Cornubian) species may be intolerant of the lower minimum temperatures in the more eastern parts of the Channel.

Turbidity may be a limiting factor in certain circumstances. The lamellibranchs, with which this paper is mainly concerned, are either deposit or suspension feeders, and in filtering off food particles may at times have to reject large quantities of inorganic material. Suspension feeders draw in water from only a few millimetres off the bottom, so that their food is qualitatively rather similar to that obtained by deposit feeders which draw in material from the surface of the sediment. Under disturbed conditions the material drawn in by these two methods of feeding must be almost identical. Large quantities of inorganic silt or clay in suspension depress the filtering rates of certain lamellibranchs (Loosanoff & Tommers, 1948; Rice & Smith, 1958), and adversely affect the development and growth of certain larvae (Davis, 1960). The high concentrations of silt and clay used by these workers would, however, only be attained in harbours and estuaries; they are probably much higher even than in water close to the bottom in open sea areas such as the Channel. Pratt (1953) showed experimentally that populations of *Venus mercenaria*, a suspension feeder, grew 24% faster in sand than in an adjacent plot of sandy mud containing much organic matter. On the whole one may expect high concentrations of silt and clay to affect suspension feeders more adversely than deposit feeders, since the latter are normally accustomed to sort their food from deposits containing a high proportion of non-edible material.

Shelter from excessive bottom-disturbance is a condition of survival for many forms. The densest populations usually occur in the more sheltered areas, but in assessing the effect of wave or current disturbance both bottom contour and depth must be taken into account. In general the western end of the Channel is more scoured by waves and the more eastern areas by tidal currents. Rich populations occur in bays, not only because of the shelter they provide but also because of the presence of eddies which appear to favour the

settlement of larvae (Orton, 1937) and the deposition of food in the form of detritus.

Many common benthos species appear to be tolerant, within limits, of a fairly wide range of sediment grades (e.g. Davis, 1925)—otherwise they would not be so generally distributed. Subtle physico-chemical or biological factors such as were found by Wilson (1958) to affect the settlement of *Ophelia* larvae could scarcely be necessary for the settlement of species apparently more catholic in their choice of bottom.

On the other hand, Wilson & Armstrong's (1958) experiments on the viability of certain larvae in different natural waters suggest the importance of as yet unknown factors in sea water which may affect the development of certain species.

It is very difficult to assess the relative importance of the different factors, as outlined above, in controlling the distribution of species. Certain factors (such as salinity in the Channel) appear not to vary sufficiently to be important, but the possibilities of the interaction of two or more such factors cannot be overlooked. It is in fact easy to say that a certain distribution is not apparently correlated with one particular factor, but where, as is generally the case, so many factors may be interacting, one can do no more than suggest possible or probable causes.

NATURE OF THE FAUNISTIC BOUNDARIES

Faunistic boundaries in the Channel appear to fall into two categories: the rigid boundaries to the Western and West Channel species, beyond which living specimens or dead shells are seldom taken, and the more indefinite boundaries shown by a gradual decline as species approach the limits of their distribution. The principal distribution patterns in the Channel are summarized in Fig. 15.

In discussing faunistic boundaries the question arises as to whether the populations occurring near their limits of distribution are self-maintaining, or whether they are a collection of non-breeding individuals recruited by larval drift (or even by migration of adults) from areas more favourable to reproduction of the species. Little information is available on this point, the most pertinent data being those of W. J. Rees (1950) and Rees & Lumby (1954) which indicate that populations of *Octopus vulgaris* on the south coast of England are mainly derived from larvae drifted across the Channel from the Channel Islands—S. Malo region, the octopus only breeding in English waters in certain years. The larval life of the octopus is fairly long, however, estimated at 2 months, which is probably rather longer than that of the majority of pelagic larvae. For the invertebrates studied in the dredge survey, particularly the critical Western species, however, information on breeding seasons, the existence of larvae and the duration of larval life are insufficient to assess the possible importance of larval drift (Thorson, 1946; C. B. Rees, 1951, 1954).

Fig. 15. Distribution patterns on the English side of the Channel. A. Western species. The pecked line shows the maximum penetration into the Channel at any time as shown by dead shell records, the black area the present distribution. B. Cornubian species. C. West Channel species. D. West Channel species mainly confined to Great West Bay (possibly a combination of distributions shown in C and E). E. Sarnian species. F. Northern species. Occurrences outside the area covered by the survey are not shown.

Western species

Western species are at present restricted, in inshore waters, to the Cornish coast west of about Looe. There is today no apparent correlation with the distribution of *Sagitta elegans* nor with any other plankton indicator (A. J. Southward, personal communications), although there is some evidence that the occurrence of Western forms at Plymouth in the 1920's and also in the north-west North Sea is in some way connected with water of the *elegans* type. In 1922–23 the typically Western species *Myrtea spinifera*, *Gafrarium minimum*, *Tellina pygmaea*, *Gari tellinella* and *Thracia villosiuscula* were quite common in the Plymouth area, more so than anywhere off the south Cornish coast today, and *Tellina pygmaea* and *Gari tellinella* were common and *Myrtea* and *Thracia* occasional in Plymouth Sound. The farthest east limit at any time, as shown by dead shell records, appears to be the south-western part of Great West Bay. Hunt (1884) records *Gari tellinella* as common, living, off Tor Bay,

but there is no other evidence of when this maximum up-Channel penetration occurred.

The temperature régime off the north-east coast of Scotland, where some Western species occur, is several degrees lower than off Plymouth. The German charts (Deutschen Seewarte Hamburg, 1927) show February bottom temperatures off Scotland in the region of 5–7° C, contrasted with 8–9° C in inshore waters off Plymouth. In August the bottom temperature is 10–11° C off Scotland and about 13° C off Plymouth. Temperatures off the Scottish coast in February are comparable with those in the Straits of Dover in the same month, and in August are about 2° C cooler than anywhere in the Channel. These figures were compiled over 30 years ago, and February and August figures do not necessarily represent minimum and maximum values, but they are sufficient to show the very big differences between the two areas. On the basis of these figures the only value which might be responsible for the restriction of Western species to western areas of the Channel would be that maximum temperatures in the central and eastern areas of the Channel are too high. The slight increase in sea temperatures which has occurred in recent years would then be seen as the cause of the westward shift of their boundary since the 1920's. All five species range as far south as the Mediterranean, however, so this seems an unlikely explanation. The annual *range* of temperature off Plymouth and off Scotland is comparable, of the order of 5° C, which suggests that these might be relatively stenothermal species intolerant of the higher annual ranges in the central North Sea and English Channel. The occurrence of some Western forms in Plymouth Sound, inside the breakwater, in the 1920's appears to be critical. Conditions in Plymouth Sound tend to be estuarine, and sufficient vertical mixing probably occurs (although few data are available) for the annual range in temperature and salinity to be at least as great as in open waters in the more central and eastern parts of the Channel. This tends to discount the possibility that the species are sufficiently stenothermal (or stenohaline) for this to be a limiting factor in offshore waters of the Channel. Similar reasoning discounts the possibilities of sewage or other urban pollution, or turbidity, as limiting factors in the Channel. Pollution with kaolin and mica fragments from china clay workings occurs both in Plymouth Sound and St Austell Bay. Both *Myrtea* and *Gari tellinella* occur in St Austell Bay today, and were common in the Sound in the 1920's, so this source of pollution appears to be unimportant.

It seems quite possible that the present distribution represents the normal and that of 1922–23 the exceptional. Clark (1906) gives the following records for Cornwall:

> *Lucina* (= *Myrtea*) *spinifera*. Dead shells not uncommon, Plymouth district, but only one live specimen on Rame–Eddystone grounds (M.B.A.). Not uncommon in trawl refuse and in 20- to 30-fathom dredgings from Falmouth Bay; Mount's Bay; St Mary's Sound, Scilly.
>
> *Tellina pusilla* (= *pygmaea*). Not recorded east of Fowey. (Fowey is at the eastern end of St Austell Bay).

The apparently abrupt nature of the boundary both in the English Channel and North Sea suggests the action of an injurious factor acting at some stage earlier than at maturity of the adult. A factor which merely prevented reproduction would tend to give a more indefinite boundary, owing to larval drift, as in the case of the octopus. That larvae do drift beyond the area occupied by adults is suggested by C. B. Rees (1951, 1954), who recorded on several occasions in autumn larvae believed to be of *Myrtea spinifera* in the central North Sea. If this is the normal state of affairs it suggests that limiting factors operate either just before, during or just after metamorphosis. As to the nature of this limiting factor one can only point to the possibility that some trace substance or 'metabolite' is responsible.

West Channel species

West Channel species also have a fairly rigid eastern boundary which suggests that larval drift may not be a significant factor in distribution. The number of records of dead shells in inshore waters east of St Alban's Head is negligible, suggesting that the boundary is fixed in position, and since it lies near the geographical division between the western and eastern halves of the Channel it is likely that it is in some way connected with the abrupt change in the width and character of the Channel east of a line from Portland Bill to Cap de la Hague. The unfavourable nature of the grounds in Poole Bay for certain species and the 'rough' tide-swept bottom south of the Isle of Wight may tend to over-emphasize the sharpness of the faunistic boundary, but it is significant that it occurs for so many species, with such a variety of sediment preferences, at about the same point. Of the species confined to the western half of the Channel, a few are at the northern end of their range, and these have been included in the Cornubian group. The majority of West Channel species range well north and south of the British Isles, and it seems unlikely that temperature limits them to the western Channel. The majority of the lamellibranchs are suspension feeders, which suggests that the increased turbidity in the eastern Channel, due to stronger tidal streams and rather shallower water, may partly account for their absence. Many West Channel species are, however, generally distributed in the North Sea, and *Venus striatula*, a species absent from the eastern Channel, is common off the Dutch coast where the bottom fauna has the following characteristics (Kristensen, 1959): 'they all thrive on rather fine as well as on rather coarse sand, they tolerate salinity fluctuations between at least 30 and 35 ‰, and they can stand the high summer temperatures of 17° C and the low winter temperatures of 2° C and even less.' Such an environment, evidently much more rigorous than in the eastern Channel, scarcely accounts for the absence of *V. striatula* in the latter area. Another West Channel species, *Dosinia exoleta*, also occurs, in lesser numbers, off the Dutch coast.

Turbidity may be the limiting factor in the eastern Channel, but it is

difficult to believe that this could affect the adults of species burrowing in muddy deposits, such as *Turritella* and *Amphiura filiformis*. Such species must at times have to survive in water which is very turbid near the bottom, whatever the condition of the overlying water mass. The eastern end of the Channel, particularly Rye Bay, supports a rich fauna of sand and muddy-sand dwellers, including a number of suspension-feeding lamellibranchs. Much more information is required on the degree of turbidity of waters in the immediate neighbourhood of the bottom and the ability of suspension feeders to withstand such conditions before we can fully assess the importance of this factor.

Both the Western and West Channel echinoderms seem to penetrate slightly farther east than the lamellibranchs. *Ophiocomina nigra* occurs at least as far east as Plymouth Sound (Marine Biological Association, 1957), and there are isolated records of other possibly western species (e.g. *Amphiura chiajei*, *Amphiura securigera*, *Pseudocucumis mixta*) up to Bigbury Bay. Similarly, *Amphiura filiformis* crosses the east/west Channel boundary, the last records being in Poole Bay. Information on the echinoderms is so much less complete than on the molluscs that one can do no more than remark on this apparent anomaly.

At this point a comparison with Crisp & Southward's (1958) conclusions on the shore fauna may be of value. They found that a number of species were limited on the English side to the coastline west of the Isle of Wight, and that the majority of these were southern species with northern limits in the British Isles. Of the species ranging well to the north of the British Isles, the majority were found in similar abundance in both halves of the Channel. This is almost the reverse of the situation with the offshore fauna, in which the majority of southern species (the Sarnian group) occurred in both halves of the Channel, and the species restricted to the western Channel were in the main not near their northern limits. For this reason I am less inclined to view temperature as an important limiting factor than were Crisp & Southward for the intertidal fauna.

Cornubian species

The numbers of Cornubian species are small, and their distributional boundaries indistinct. They are all southern species spreading into the Channel from the west, and their typical habitat is off the south Cornish coast, their numbers decreasing fairly rapidly on passing up-Channel. Absence of low winter temperatures may be a limiting factor, but as many occur at L.W.S.T. as well as offshore in Cornwall they are probably not highly stenothermal. The boundaries limiting the Western and West Channel groups may affect these species also, but to a less marked extent. Thus *Tellina squalida* becomes less common offshore and very rare on the shore from about Plymouth eastward. Hepper (1957) found that the southern species *Mytilus galloprovincialis* reached its eastern limit in the Channel at about Plymouth, and

this may be due to a change in the character of the water masses affecting a species already nearing its limits of temperature tolerance. Other Cornubian species range farther east, *Labidoplax digitata* extending nearly to Portland, and its final limits may be at the east/west Channel boundary.

Sarnian species

The Sarnian species are all southern forms nearing their northern limits in the English Channel. As with the Cornubian species their extreme limits are rather indefinite, and they may extend into the North Sea along the Belgian and Dutch coasts and also into Scandinavia. On the English side of the Channel Sarnian forms occur mainly in the central regions, their presence depending probably on sufficiently high summer temperatures to enable spawning to occur, and they also seem to be tolerant of the lower winter temperatures in the same areas. Successive stages in a possible northward spread of southern forms into the Channel may be traced from a study of the present distribution of southern species from the Bay of Biscay northward. The most southerly forms are restricted to the South Brittany coasts, occurring in such areas as Quiberon Bay, and farther north in Douarnenez Bay (Prenant, 1932, 1939, and my own observations). Examples are *Donax trunculus* and *Labidoplax thomsoni*. To the north the north-western corner of Finistère is surrounded by water which reaches only about 15° C at the surface in summer (Lumby, 1935; Crisp & Fischer-Piette, 1959), and which may therefore act as a partial barrier to further extension of shallow-water forms into the Channel. The Channel Islands–S. Malo region is warmer in summer than farther west, and species which reach their limit in this area may be commoner than at Roscoff in north-west Brittany. An example is the ormer, *Haliotis tuberculata*, which does not occur in Britain. The next stage is represented by *Octopus vulgaris* which breeds on the south side of the Channel, and is represented by a normally non-breeding population off the south coast of England. A stage further takes us to species like *Dentalium vulgare* and *Nassarius pygmaeus*, which are quite common in the central Channel on the English side, and may well form a self-supporting population, although there is no evidence on this point. West of Start Point they become very rare, perhaps because of lower summer temperatures. *Venus verrucosa*, most abundant in the central region, occurs both at low tide and offshore west of Start Point (e.g. Plymouth Marine Fauna, 1957; Clark, 1906), but is never particularly common there. It represents a further stage in colonization of the English coast, the final stage being shown by *Venerupis rhomboides*, a southern form common throughout the length of the Channel.

This series is hypothetical, since we know little of the limiting factors for individual species, and indeed the observations of Rees and Lumby (1954) failed to show a clear correlation between the breeding of *Octopus vulgaris* and sea temperature, but it suggests the most probable route for colonization

of the Channel by many southern forms. Two successive barriers to such a northward spread exist: the first the cooler summer temperature off the tip of Finistère, the second the deep and probably rather barren area between the Channel Islands and the south coast of England. To what extent these barriers can be regularly bridged by larval drift is uncertain.

Northern species

This last group includes only a few species. One northern species, *Chlamys tigerina*, appears to be restricted to the western end of the Channel, but the other northern species, *Spisula elliptica*, *Buccinum undatum* and *Maxmulleria lankesteri* show a decidedly eastern trend. *Buccinum* spawns in winter and early spring at Plymouth, as one would expect of a species at the southern end of its range. The distribution of *Buccinum* both in the Channel and in respect to its southern limits in the Bay of Biscay is rather similar to that of *Balanus balanoides* (Crisp & Southward, 1958; Crisp & Fischer-Piette, 1959).

The tendency for both *Spisula* and *Buccinum* to occur in much greater numbers in the central and eastern parts of the Channel may well be related to the lower winter temperatures in these areas.

Criticism of this paper will no doubt be levelled at the often-deplored attempt to group species into ecological categories. This has only been possible by ignoring the more detailed distribution of species, no doubt largely due to specific preferences for sediments of a particular grade, and at the same time assuming that a sufficient range of types of sediment occurs in each area to provide a suitable habitat for the commoner species. In this way an attempt has been made to use species as indicators of hydrographical conditions. It is at once clear that being fixed in the adult stage, their distribution has a different order of significance compared with plankton indicators. There are some indications that the distribution of some groups is not so much connected with the dispersal of pelagic larvae, but with the conditions of life experienced either at metamorphosis or in the early bottom stages. Thus they may well serve as more permanent indicators of the hydrographic conditions in particular areas, and so supplement the information derived from plankton indicators.

SUMMARY

A survey has been made of the bottom fauna in *ca.* 10–50 m along the south coast of England, using a modified anchor-dredge. Particular attention has been paid to the molluscs (both living and dead) and to the echinoderms, only larger or more conspicuous members of other groups being identified.

Within the Channel there are considerable variations in temperature and other hydrographic conditions, and these are discussed in relation to their possible role as limiting factors in distribution. The fauna and bottom deposits of the different areas covered in the survey are briefly described.

Distribution trends are classified as:

(1) Species generally distributed in the Channel. This is the largest group, and includes three species reaching their northern limits in British waters.

(2) Species confined to the western parts of the Channel.

(*a*) *Western species*. Four or five species common at Plymouth in the 1920's, but now absent or very rare. Small numbers are found today only off the south Cornish coast, and those which occur in the North Sea are restricted to the north-western areas. Apart from one species, these forms are not at their geographical limits in the English Channel, and their occurrence may be partly related to the presence of 'western', but not necessarily *Sagitta elegans*, water.

(*b*) *Cornubian species*. These are near the northern end of their range, and occur quite commonly off the south Cornish coast, becoming progressively less common farther up-Channel. It is suggested that their distribution is related to an intolerance of the lower winter temperatures in the more eastern part of the Channel.

(*c*) *West Channel species*. These are generally distributed in the western half of the Channel, but suddenly disappear on entering the eastern Channel. This group comprises many species generally distributed and common in British and northern European waters. Since the majority are not near their geographical limits, temperature is discounted as a limiting factor, and a possible explanation of their disappearance is the greater turbidity of waters in the eastern Channel.

(3) Species distributed around the central region of the Channel. This includes a number of species at the northern end of their range which are common in the Channel Islands–S. Malo area, and are therefore referred to as *Sarnian* forms. Although commonest in the central Channel, many are present, in smaller numbers, along the Devon and Cornish coasts west of Start Point. Their abundance in the central region may be due to the higher summer temperatures in this area, while at the same time they appear to be tolerant of the relatively low winter temperature in the same places.

(4) *Northern species*, most common in the eastern Channel, gradually decreasing in numbers farther west. Probably dependent on low winter temperatures.

Distributional boundaries are of two kinds: the rather gradual boundaries to the Cornubian, Sarnian, and Northern species, which are believed to be due to temperature; and the abrupt boundaries of the Western and West Channel species ascribed to as yet unknown hydrographic factors.

A special type of 'submergence', similar to that described by Ursin for echinoderms, is described in relation to lamellibranch faunas in the Channel.

An analysis of the distribution of different phyla and classes has been made. The lamellibranchs show a fair balance between the trends outlined above, the gastropods do not contain any species in the Western group but are

represented by a number of Sarnian forms, so that there are more species in the central Channel than in western areas. The echinoderms have a predominantly western distribution, in a general sense, and numbers of species show a decline on passing up-Channel.

Total populations of individuals in different areas of the Channel are considered. The richest areas are around Falmouth (also the richest in numbers of species), around Portland and in Rye Bay. The total in each of these areas is much the same, so that there is no evidence that the eastern Channel is any poorer in terms of the 'standing-crop' than the more western areas.

The role of dead shells as evidence of present and past populations is briefly considered. There is no evidence that dead shells are transported any appreciable distance from the areas in which they originally lived.

Possible limiting factors are discussed. Temperature, turbidity, shelter from bottom-disturbance, and the effects of different sediments on distribution are considered, and the difficulties in assigning any single factor as causative are stressed.

The possible causes of the different distributional trends are discussed, with special reference to the Western, West Channel and Sarnian species. A number of possible factors are discounted, but the causes of the limited distribution of these two groups remain obscure.

The Appendix contains a note on the identity of specimens of *Spisula* used by the late Dr D. Atkins in her work on ciliary mechanisms.

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APPENDIX

STANDARDIZATION OF TERMS USED IN THIS PAPER

The *Plymouth area* stretches along the coast from Looe to Bolt Tail, at the eastward end of Bigbury Bay, and extends seawards within a radius of about 20 miles from Plymouth. Records from Salcombe are here included in the Plymouth area, except in Table 8.

Western and *eastern* halves of the *Channel* lie on either side of St Alban's Head. The term *central Channel* is loosely applied to the middle parts of the length of the Channel. *Mid-Channel* refers to the central east-west axis of the Channel (i.e. to 'mid-stream').

FAUNA LIST

Although I am indebted to a number of authorities for checking or identifying certain species, the list is in the main confined to those with which I am personally familiar. This was particularly important for the molluscs where it was necessary to sort through large quantities of sievings to check dead shell records.

The coverage of different groups is as follows:

Lamellibranchs, scaphopods and echinoderms—as complete as possible.

Gastropods—fairly complete, so far as living specimens go, but records of species taken only as dead shells are generally omitted.

Crustaceans—mainly burrowing species only. The sieve mesh was too large to take many amphipods, and these have not been included.

Other groups—confined to a few larger or more conspicuous species.

Specimens identified to genus only are not to be considered as species additional to those of the same genus in which specific identity is given. Thus records of *Natica* sp. are probably all either *N. catena* or *N. alderi*.

In the list of living specimens, station number is followed by numbers of individuals, in parentheses.

Dead shells are usually recorded as the number of *stations* at which each species was recorded in each of the fourteen areas. For example, 2–8 means records at 8 stations in area 2. The few stations not included in these areas are also included in the list. For certain more critical species numbers of dead shells at each station are recorded. These are rather approximate, but are based on the following frequency code:

	Lamellibranchs (nos. of valves)
A (abundant)	Several hundred
C (common)	> 32
F (frequent)	17–32
O (occasional)	9–16
R (rare)	1–8

+, present; ?, identity queried.

When living specimens were taken, dead shells are not recorded from the same station.

Nomenclature follows the Plymouth Marine Fauna (Marine Biological Association, 1957). Other species are listed by Winckworth (1932) and Stephen (1960).

PHYLUM MOLLUSCA

Class Lamellibranchia

Nucula nucleus (L.)
Living: 2 (2), 3 (4), 7 (12), 11 (14), 14 (1), 17 (85), 18 (1), 20 (2), 22 (8), 35 (2 ?), 36 (40), 39 (1), 48 (2), 54 (88), 64 (11), 79 (1), 98 (1). Dead shells not recorded.

N. hanleyi Winckworth
Living: 7 (1), 48 (1), 58 (1), 61 (9), 74–1 (22), 74–2 (2), 81 (2), 82 (11), 83 (2), 85 (1), 91 (1 ?), 104 (3), 120 (13), 123 (7), 133 (1), 134 (1), 135 (3), 137 (9), 163 (1). Dead shells not recorded.

N. turgida Leckenby & Marshall
Living: 1 (5), 4 (39), 12 (2), 18 (2), 19 (51), 20 (5), 32 (8), 42 (5), 44 (3), 50 (1), 51 (6), 52 (14), 53 (120), 66 (18), 67 (17), 68 (13), 69 (3), 70 (1), 71 (36), 73 (2), 77 (1), 79 (53), 105 (14), 110 (1), 124 (1), 127 (6), 136 (2), 140 (14), 141 (9), 159 (11), 163 (1), 166 (2), 169 (12), 170 (1). Dead shells not recorded.

Glycymeris glycymeris (L.)
Living: 7 (2), 74–1 (4), 135 (2), 138 (9). Dead: 1–6, 2–8, 3–1, 4–6, 5–3, 7–1, 8–3, 9–3, 10–1, 14–2. Stn. 120.

Pinna fragilis Pennant
Dead: 104 (1).

Chlamys tigerina (Müller)
Dead: 1–3, 2–8, 3–5, 4–6, 5–2, 7–1. Stn. 120.

C. opercularis (L.)
Living: 4 (1), 41 (1), 57 (6), 82 (1), 85 (2), 86 (2), 98 (2), 101 (2), 102 (2), 154 (1), 156 (1). Dead shells were recorded at nearly every station.

C. varia (L.)
Living: 26 (1), 55 (2), 64 (1), 85 (2), 91 (1), 95 (8), 96 (1), 97 (2), 98 (4), 100 (2), 101 (4, 1 ?), 167 (3). Dead: 2–1, 3–1, 4–4, 5–5, 7–2, 8–3, 9–7, 10–6, 11–8, 12–9, 13–9, 14–9. Stns. 65–2, 74–2.

C. distorta (da Costa)
Living: 86 (1), 98 (1), 167 (1). Dead: 1–2, 2–7, 3–4, 4–3, 5–6, 6–2, 7–2, 9–1, 12–3, 13–7, 14–3.

Pecten maximus (L.)
Living: 56 (1), 158 (1), 163 (2). Dead: 1–4, 2–6, 3–4, 4–4, 5–1, 6–1, 8–2, 11–3, 12–2, 13–3, 14–2. Stn. 120.

Astarte sulcata (da Costa)
Dead: 133 (2), 139 (2).

Cyprina islandica (L.)
Living: 42 (1), 46 (1). Dead: 1–1, 2–1, 3–2, 7–3, 8–2, 10–1. Stn. 120.

Diplodonta rotundata (Montagu)
Living: 48 (2), 56 (1), 62 (1), 78 (2), 81 (1), 103 (1), 104 (2), 112 (12), 120 (4), 129 (5), 133 (1), 148 (1), 153 (1). Dead: 1–4, 2–4, 3–3, 4–4, 5–3, 6–1, 7–3, 8–4, 11–2, 12–5, 13–4, 14–5. Stns. 74–2, 120.

Thyasira flexuosa (Montagu)
Living: 4 (1), 12 (4), 18 (2), 19 (1), 32 (6), 44 (1), 49 (6), 50 (2), 51 (3), 52 (1), 53 (4), 77 (1), 79 (1), 124 (3), 140 (4), 169 (1), 170 (3). Dead: 1–4, 2–4, 3–2, 4–3, 5–4, 6–5, 7–3, 8–4, 14–1. Stns. 79, 120.

Lucinoma borealis (L.)
Living: 1 (1), 120 (1), 127 (2), 136 (1), 140 (2), 160 (1). Dead: 4 (1), 82 (1), 117 (3), 119 (1), 120 (O), 121 (1?), 123 (1), 127 (8), 129 (1), 136 (3), 140 (1), 141 (5), 151 (4), 166 (1).

Myrtea spinifera (Montagu)
Living: 119 (1), 126 (2). Dead: 110 (13), 113 (2), 117 (17), 120 (1), 135 (4), 154 (2), 171 (1), 172 (11).

Lepton squamosum (Montagu)
Living: 18 (1), 117 (2). Dead: 2–2, 3–4, 4–3, 5–1, 6–3, 7–4, 8–4, 11–1, 12–2, 13–1, 14–1. Stn. 120.

Mysella bidentata (Montagu)
Living: 12 (1), 18 (1), 32 (1), 50 (2), 51 (1), 52 (2), 53 (7), 71 (1), 79 (3), 124 (5), 141 (2). Dead: 1–2, 2–2, 3–2, 6–4. Stn. 79.

Montacuta substriata (Montagu)
Commensal with *Spatangus purpureus*. Living: 88 (2).

M. ferruginosa (Montagu)
Living: 49 (1), 51 (1), 53 (1), 60 (5), 67 (2), 105 (1), 119 (1), 126 (1), 169 (3). Dead: 1–2, 2–4, 3–1, 4–1, 5–4, 6–6, 7–5, 8–1, 12–1, 13–2, 14–2. Stn. 79.

Laevicardium crassum (Gmelin)
Living: 48 (1), 83 (2), 102 (1), 123 (1), 135 (2), 137 (2), 138 (1), 148 (2), 154 (3), 156 (1), 157 (3). Dead: 1–4, 2–9, 3–4, 4–5, 5–2, 6–1, 7–2, 8–4, 9–2, 11–4, 12–6, 13–9, 14–6. Stns. 37, 65–2, 74–2, 120.

Cardium echinatum L.
Living: 4 (2), 5 (1), 18 (1), 32 (2), 66 (1 ?), 67 (1 ?), 71 (1), 75 (1), 76 (1), 79 (1), 103 (1), 104 (1), 105 (1), 107 (1), 116 (1), 118 (1), 124 (1, 1 ?), 136 (1, 2 ?), 141 (1), 150 (2), 151 (1), 160 (2). Dead: 1–4, 2–3, 3–6, 4–3, 5–5, 6–6, 7–6, 8–4, 10–3, 12–4, 13–3, 14–7. Stns. 32, 79, 120.

Gouldia minima (Montagu)
Living: 134 (1), 137 (1), 138 (1). Dead: 112 (2), 113 (11), 116 (2), 120 (9), 123 (12), 127 (5), 129 (1), 130 (1), 133 (8), 134 (18), 135 (13), 136 (1), 137 (10), 138 (5), 139 (12), 154 (9), 155 (3), 157 (13).

Callista chione (L.)
Dead: 117 (1), 120 (C), 122 (1), 126 (1), 136 (O), 138 (1), 141 (1).

Dosinia exoleta (L.)
Living: 81 (1), 121 (1), 130 (1), 137 (3), 138 (15). Dead: 1–1, 2–8, 3–3, 4–2, 5–1, 7–1, 8–3, 9–1.

D. lupinus (L.)
Living: 45 (1), 104 (2), 105 (2), 116 (2), 117 (1), 119 (6), 120 (74), 123 (2), 124 (1), 127 (13), 129 (4 ?), 136 (1), 140 (4), 141 (9), 150 (1), 152 (3), 153 (11), 159 (1). Dead: 1–7, 2–6, 3–6, 4–3, 5–5, 6–3, 7–2, 8–1. Stn. 120.

Dosinia sp.
Living: 140 (1). Dead: 1–1, 2–1, 3–1, 4–1, 7–2, 8–2.

Venus verrucosa L.
Living: 2 (5), 3 (5), 10 (1), 25 (6), 26 (3), 27 (11), 28 (2), 29 (3), 30 (2), 37 (2), 39 (2), 40 (10), 54 (1), 55 (1), 97 (18), 98 (2), 100 (3), 101 (1). Dead: 2–3, 4–1, 8–1, 9–5, 10–3, 11–2, 12–1. Stns. 22, 28, 29, 36, 40.

V. casina L.
Living: 9 (2), 10 (2), 137 (1). Dead: 1–3, 2–4, 3–2, 8–1, 9–3.

V. ovata Pennant
Living: 5 (1), 7 (7), 11 (1), 35 (3), 36 (1), 37 (2), 39 (1), 48 (148), 49 (17), 55 (1), 56 (2), 91 (7), 92 (1), 101 (1), 102 (7), 159 (1), 163 (2). Dead shells occurred in all areas.

V. fasciata (da Costa)
Living: 102 (1), 113 (1), 128 (2), 134 (1), 137 (4), 138 (9), 163 (1). Dead: 1–6, 2–8, 3–7, 4–6, 5–1, 7–1, 8–1, 9–2, 13–1 (1 valve only). Stn. 120.

V. striatula (da Costa)
Living: 1 (3), 4 (11), 5 (1), 41 (3), 42 (2), 44 (2), 45 (14), 46 (3), 49 (8), 50 (1), 51 (4), 52 (2), 53 (8), 105 (7), 110 (4), 120 (1), 136 (32), 140 (2), 141 (24), 152 (1), 163 (2), 166 (1), 170 (1), 171 (1), 173 (1). Dead: 1–7, 2–4, 3–8, 4–6, 5–8, 6–10, 7–8, 8–5. Stn. 120.

Venerupis rhomboides (Pennant)
Living: 2 (2), 3 (2), 4 (1), 5 (4), 7 (3), 9 (3), 10 (2), 16 (1), 17 (10), 20 (1), 22 (5), 25 (35), 26 (23), 27 (39), 28 (30), 29 (15), 30 (11), 35 (3), 36 (25), 37 (9), 39 (6), 40 (44), 47 (3), 48 (3), 55 (5), 56 (9), 57 (13), 58 (22), 59 (1), 64 (1), 83 (1), 85 (5), 90 (4), 91 (3), 92 (3), 97 (3), 98 (7), 100 (1), 101 (1), 102 (4), 103 (2), 104 (2), 121 (1), 122 (2), 134 (1), 137 (6), 138 (18), 154 (1), 155 (1), 163 (1). Dead shells occurred in all areas.

V. pullastra (Montagu)
Living: 17 (1), 31 (3), 36 (1). Dead: 17 (2), 35 (2), 64 (3).

Venerupis aurea (Gmelin)
Living: 2 (19), 3 (14). Dead: 1 (6).

Mysia undata (Pennant)
Living: 1 (3), 4 (1), 18 (1), 41 (3), 42 (1 ?), 43 (1), 44 (2), 51 (1), 82 (1), 83 (1), 91 (1), 105 (1), 106 (1), 109 (1), 110 (2), 119 (1), 140 (1), 160 (1). Dead: 1–3, 2–5, 3–3, 4–4, 5–2, 6–4, 7–6, 8–5, 9–1, 10–1, 12–6, 13–3, 14–2. Stns. 74–2, 120.

Spisula subtruncata (da Costa)
Living: 1 (25), 4 (130), 5 (1), 18 (9), 20 (22), 21 (1), 32 (2), 35 (1), 41 (1), 42 (25), 43 (27), 44 (9), 48 (3), 49 (2), 52 (16), 53 (15), 105 (5), 136 (2), 166 (5), 169 (6). Dead: 1–5, 2–3, 3–3, 4–1, 5–8, 6–8, 7–8, 8–5, 10–4, 12–3, 13–1, 14–7. Stns. 32, 79, 120.

S. solida (L.)
Living: 165 (2), 168 (12). Dead: 2–2, 10–1 ?. Stn. 28.

S. elliptica (Brown)
See Van Urk (1957). Living: 8 (1), 9 (2), 10 (2), 15 (1), 24 (2), 34 (1), 36 (1), 38 (1), 56 (2), 58 (1), 60 (1), 61 (2), 62 (1), 63 (1), 65–2 (1), 67 (1), 72 (2), 74–1 (2), 82 (2), 83 (1), 91 (4), 98 (3), 99 (1), 102 (1), 165 (1). Dead: 1–2, 2–2, 3–2, 4–3, 5–5, 6–1, 7–3, 8–2, 9–4, 10–3, 11–7, 12–9, 13–10, 14–10. Stns. 24, 37, 38, 39, 65–2, 74–2, 79, 120.

Spisula sp.
Dead: 62 (1), 67 (1).

Mactra corallina (L.)
Living: 4 (1), 42 (1 ?), 43 (8), 44 (1), 66 (3), 67 (1), 68 (2), 150 (2), 159 (1), 169 (1). Dead: 2–2, 3–2, 4–1, 5–4, 6–1, 7–1, 8–4, 10–3, 12–2, 13–1, 14–8.

Lutraria lutraria (L.)
Living: 18 (1), 45 (2), 50 (3), 51 (3), 52 (2), 53 (3), 76 (1), 107 (1), 140 (2), 151 (4). Dead: 2–3, 3–3, 4–1, 5–3, 6–4, 7–3, 10–1. Stn. 120.

L. angustior Philippi
See Holme (1959). Living: 122 (1), 137 (2), 138 (1), 139 (1), 157 (1). Dead: 1–2, 2–7, 3–4, 4–4, 5–2, 7–1, 8–5, 9–4, 11–1, 13–3, 14–2. Stns. 26, 40, 120.

L. magna (da Costa)
Dead: 7 (2); 26 (*ca.* 6), 27 (12), 38 (1), 40 (1), 61 (1).

Lutraria sp.
Living: 141 (4). Dead: 157 (1).

Gari depressa (Pennant)
Dead: 136 (1).

G. costulata (Turton)
Living: 137 (1); 157 (1). Dead: 123 (1), 133 (1), 134 (1), 135 (7), 138 (1), 157 (1).

G. fervensis (Gmelin)
Living: 41 (4), 42 (3), 44 (3), 61 (1), 71 (1), 72 (1), 82 (1), 104 (2), 105 (4), 120 (3), 127 (1), 136 (1), 141 (1), 150 (2), 166 (1). Dead: 1–6, 2–5, 3–4, 4–2, 5–4, 6–3, 7–6, 8–4, 9–1, 10–1, 12–3, 13–1. Stn. 120.

G. tellinella (Lamarck)
Living: 120 (2), 123 (3), 157 (1). Dead: 55 (2), 62 (1 ?), 92 (1), 102 (1), 103 (1), 113 (F), 120 (O), 123 (F), 127 (2), 128 (1), 133 (2), 134 (7), 135 (C), 136 (O), 137 (5), 139 (6), 148 (1), 151 (1), 154 (F), 157 (F), 159 (1), 163 (1), 165 (1).

Solecurtus chamasolen (da Costa)
Living: 108 (2), 109 (3), 110 (1), 116 (1), 117 (1), 119 (2), 124 (4), 126 (4), 152 (1), 153 (1), 173 (5). Dead: 1–3, 2–1, 3–5, 4–2, 6–3, 7–2, 8–1. Stn. 120.

S. scopula (Turton)
Dead: 1–1, 2–4, 3–1, 4–3, 14–1 (1 valve only).

Abra prismatica (Montagu)
Living: 60 (*ca.* 36), 61 (*ca.* 11), 65–1 (4, 1 ?), 68 (1), 70 (1), 73 (5), 74–1 (1), 88 (3), 91 (1), 120 (4), 141 (2), 164 (1). Dead: 60 (C), 61 (O), 62 (1), 63 (R), 65–1 (O), 65–2 (2), 67 (3), 68 (5), 69 (2), 70 (1), 71 (O), 72 (R), 73 (5), 78 (3), 81 (1), 82 (12), 83 (5), 87 (1), 88 (F), 89 (1), 90 (1), 91 (1), 103 (1), 104 (2), 105 (1), 120 (9), 124 (1), 126 (8), 127 (3), 129 (1), 136 (4), 141 (3).

A. alba (W. Wood)
May include a few *A. nitida* (Müller), which were not distinguished with certainty. Living: 1 (16), 4 (177), 20 (2), 22 (24), 41 (2), 48 (5), 51 (4), 52 (2), 53 (4), 60 (*ca.* 31), 64 (15), 66 (*ca.* 55), 67 (56), 68 (7), 69 (3), 70 (1), 71 (15), 72 (1), 73 (26), 74–1 (1), 76 (1), 77 (*ca.* 57), 79 (57), 83 (1 ?), 91 (3), 104 (3), 105 (34), 112 (2), 120 (2), 140 (3), 159 (4), 163 (1), 166 (2), 169 (1), 170 (1). Dead shells in all areas except 9.

Arcopagia crassa (Pennant)
Living: 123 (1), 135 (3), 138 (1), 155 (2), 157 (1), 161 (1). Dead: 1–4, 2–8, 3–3, 4–5, 7–1, 9–1. Stn. 120.

Tellina donacina L.
Living: 48 (1), 56 (1), 75 (1), 81 (1), 83 (1), 85 (2), 91 (3), 102 (3), 112 (3), 116 (1), 120 (20), 128 (1), 134 (1), 135 (2), 161 (1). Dead: 1–3, 2–4, 3–3, 4–5, 5–3, 6–1, 7–2, 8–3, 9–2, 11–3, 12–7, 13–8, 14–6. Stns. 74–2, 120.

T. pygmaea Lovén
Living: 113 (1), 120 (2). Dead: 82 (1), 83 (1), 120 (8), 123 (8), 135 (1), 136 (1), 157 (2).

T. fabula Gmelin
Living: 12 (16), 19 (3), 50 (6), 52 (1), 53 (4), 60 (70), 66 (1), 67 (16), 68 (10), 69 (7), 70 (1), 71 (21), 73 (19), 136 (97), 140 (1), 141 (106), 169 (2). Dead: 1–1, 2–3, 3–2, 4–2, 5–1, 6–5, 7–5, 8–2, 10–4, 12–4, 13–3, 14–7. Stns. 79, 120.

T. tenuis da Costa
Living: 33 (1), 72 (1). Dead: 13–2, 14–3.

T. squalida Montagu
Living: 136 (2), 141 (4). Dead: 1 (2), 4 (R), 50 (3), 117 (4), 120 (1), 136 (10), 140 (1), 141 (8).

Donax vittatus (da Costa)
Dead: 5–1, 7–1, 12–1, 14–5.

Phaxas pellucidus (Pennant)
Living: 1 (1), 4 (20), 41 (5), 42 (12), 43 (19), 44 (45), 45 (3), 48 (8), 49 (1), 52 (2), 53 (9), 60 (1), 63 (1), 67 (2), 70 (1), 71 (10), 73 (2), 77 (4), 104 (14), 105 (13), 111 (1), 112 (1), 113 (1), 120 (2), 124 (1), 126 (1), 127 (13), 136 (8), 140 (*ca.* 14), 141 (10), 151 (1), 153 (3), 159 (*ca.* 34), 166 (1), 173 (1). Dead: 1–3, 2–2, 3–2, 4–1, 5–3, 6–8, 7–7, 8–5, 10–1, 12–1, 13–2, 14–1. Stns. 79, 120.

Solen marginatus Pulteney
Living: 19 (1 ?), 34 (1), 35 (1). Dead: 8–1, 10–4, 12–2, 14–1.

Ensis ensis (L.)
Living: 1 (1 ?), 4 (1), 19 (2), 42 (7), 43 (3), 44 (13), 50 (10), 60 (8), 61 (1), 67 (8), 68 (*ca.* 10), 69 (9), 70 (*ca.* 26), 71 (42), 72 (8), 73 (3), 74–1 (1 ?), 105 (15), 140 (2 ?), 150 (1), 160 (1), 166 (1), 172 (1). Dead: 2–1, 3–2, 5–2, 7–4, 8–3, 10–3, 12–3, 13–4, 14–7. Stns. 32, 120.

E. arcuatus (Jeffreys)
Living: 9 (1), 28 (4), 29 (1), 83 (2), 102 (1, 1 ?), 113 (1), 135 (3), 137 (3), 165 (2). Dead: 1–1, 2–2, 4–1, 5–3, 8–2, 11–1, 12–1 ?, 13–5, 14–3. Stn. 120.

E. siliqua (L.)
Living: 50 (1 ?), 151 (1), 164 (*ca.* 3), 166 (3, 5 ?). Dead: 3–2, 5–2, 6–2.

Ensis sp.
Living: 136 (1), 166 (1). Dead: 1–1, 2–4, 3–1, 4–1, 5–3, 7–2, 9–1, 10–1, 13–1.

Saxicavella jeffreysi Winckworth
Living: 18 (24), 20 (3), 77 (69), 79 (1). Dead: 2–1, 10–2, 12–1, 14–2. Stns. 32, 79.

Corbula gibba (Olivi)
Living: 1 (1), 4 (10), 18 (1), 20 (1), 32 (1), 42 (2), 44 (2), 45 (1), 48 (2), 49 (1), 50 (1), 61 (2), 71 (9), 73 (2), 76 (1), 104 (1), 108 (1), 119 (1), 127 (1), 136 (1), 141 (2), 163 (2). Dead shells in all areas except 9 and 11.

Mya truncata L.
* Siphons only. Living: 20 (4 ? *), 64 (1), 77 (1 ? *). Dead: 3–1, 4–1, 7–2, 9–2, 10–2, 11–5, 12–4, 13–3, 14–3. Stns. 22, 26, 27 (36 (?), 39, 40.

Lyonsia norvegica Macgillivray
Living: 91 (1), 116 (1). Dead: 91 (1), 163 (1).

Pandora albida (Röding)
Living: 4 (1), 19 (1), 61 (1), 66 (1). Dead: 8–1, 10–3, 14–1. Stn. 32.

P. pinna (Montagu)
Living: 42 (2 ?), 140 (1), 159 (1), 163 (2). Dead: 2–1, 4–1, 7–1, 12–1.

Pandora sp.
Dead: 4–1, 6–1, 7–1, 12–1, 13–1, 14–1. Stns. 79, 120.

Thracia phaseolina (Lamarck)
Living: 44 (6), 45 (1), 53 (3), 119 (1), 136 (1, 2 ?), 141 (4), 166 (1). Dead: 1–3, 2–3, 3–2, 6–2, 8–1 ?. Stn. 120.

T. convexa (W. Wood)
Living: 44 (1), 108 (1), 116 (1), 152 (2). Dead: 4–1, 6–2, 7–1.

T. villosiuscula (Macgillivray)
Living: 120 (1), 123 (1), 133 (1), 138 (1). Dead: 113 (6), 120 (12), 123 (5), 134 (6), 135 (14), 136 (7), 137 (6), 138 (1), 139 (1), 141 (1), 157 (3), 159 (1).

Cochlodesma praetenue (Montagu)
Living: 112 (1), 120 (1), 127 (1), 129 (12). Dead: 120 (18), 123 (1), 127 (1), 129 (F).

Class Gastropoda

Calliostoma zizyphinum (L.)
Living: 25 (1), 54 (1), 57 (1), 64 (1), 98 (2), 100 (3), 101 (7), 167 (7). Dead: 4–1, 5–1, 6–1, 7–2, 9–3, 10–2, 11–6, 12–4, 13–3, 14–3. Stns. 40, 65–2, 74–2.

Gibbula magus (L.)
Living: 2 (2), 3 (1), 17 (1). Dead: 1–1, 2–4, 3–2, 5–1, 8–1, 9–1, 13–2. Stns. 30, 120.

Turritella communis Risso
Living: 1 (2), 4 (101), 5 (20), 46 (*ca.* 530), 47 (*ca.* 48), 48 (3), 49 (*ca.* 220), 50 (2), 51 (1), 52 (111), 53 (*ca.* 370), 119 (114), 120 (4), 170 (1), 173 (33). Dead: 1–4, 2–3, 3–4, 4–3, 5–6, 6–8, 7–8, 8–4, 10–1. Stn. 120.

Aporrhais pes-pelecani (L.)
Living: 5 (2), 166 (3). Dead: 2–2, 3–1, 5–2, 7–1. Stn. 120.

Natica catena (da Costa)
Living: 19 (1 ?). Dead: 81 (1), 120 (2), 155 (1).

N. alderi (Forbes)
Living: 4 (1), 9 (1), 19 (1), 20 (2), 42 (2), 50 (3), 54 (1), 60 (9), 64 (1), 66 (9), 67 (2), 68 (1), 69 (12), 70 (4), 71 (37), 72 (2), 73 (10), 74–1 (2), 78 (1), 79 (1), 82 (2), 102 (1), 105 (7), 119 (1), 123 (1), 127 (1), 136 (2), 140 (3), 151 (2), 159 (4), 166 (4), 169 (1). Dead: 1–2, 2–7, 3–1, 4–3, 5–3, 6–2, 7–3, 8–1, 11–1, 12–6, 13–3, 14–9. Stns. 79, 120.

Natica sp.
Living: 105 (1). Dead shells in many areas.

Calyptraea chinensis (L.)
Living: 3 (1), 4 (1), 37 (1), 48 (2), 138 (1), 163 (2). Dead: 2–2, 5–2, 6–1, 7–1, 8–1, 10–1, 11–1.

Crepidula fornicata (L.)
Living: 2 (60+), 3 (100), 4 (1), 7 (2), 11 (*ca.* 1300), 12 (3), 16 (C), 17 (36+), 19 (8), 20 (8), 22 (*ca.* 45), 24 (1), 30 (1), 37 (2), 53 (1), 57 (2), 60 (1), 64 (106), 91 (5), 93 (3), 95 (1), 97 (2), 98 (1), 163 (6), 164 (1), 167 (2). Dead: 5–2, 7–1, 8–1, 9–2, 10–7, 11–7, 12–1, 13–2. Stns. 22, 24, 27, 40.

Buccinum undatum L.
Living: 4 (1), 11 (1), 16 (1), 18 (2), 19 (6), 36 (1), 49 (1), 53 (1), 57 (1), 58 (1), 71 (3), 76 (1), 83 (1), 85 (1), 87 (1), 95 (1), 98 (1), 101 (1), 102 (1), 168 (5). Dead: 3–1, 5–2, 6–1, 7–1, 8–4, 10–2, 11–3, 12–4, 13–2, 14–1. Stn. 120.

Nassarius reticulatus (L.)
Living: 2 (6), 3 (4), 4 (3), 11 (4), 12 (1), 18 (1), 19 (12), 20 (2), 24 (3), 52 (2), 53 (2), 60 (7), 64 (1), 66 (9), 67 (10), 68 (10), 69 (7), 70 (7), 71 (23), 72 (1), 73 (3), 74–1 (3), 88 (1), 136 (1), 150 (2), 151 (12), 159 (1), 160 (3), 163 (6), 166 (2), 167 (3), 168 (1), 171 (1), 172 (1). Dead: 2–2, 3–2, 4–2, 5–4, 7–3, 8–1, 9–1, 10–2, 11–1, 12–5, 13–3, 14–5. Stns. 30, 35, 40, 79.

N. incrassatus (Ström)
Living: 2 (1), 57 (1), 85 (1 ?), 91 (1), 101 (4), 134 (1). Dead: 2–4, 3–4, 4–2, 5–2, 6–2, 7–1, 8–1, 9–1, 10–2, 11–2, 12–5, 13–6, 14–3.

N. pygmaeus (Lamarck)
* May include some dead shells. Living: 1 (8*), 4 (56*), 18 (1), 19 (1), 20 (1 ?), 44 (1), 46 (6), 48 (1), 49 (5), 50 (7), 52 (2), 53 (2), 169 (3). Dead: 3–2, 4–1, 5–2, 6–3, 7–4, 8–2, 10–2, 12–1, 13–1, 14–5. Stn. 79.

Nassarius sp.
Dead: 89 (1).

Cylichna cylindracea (Pennant)
Living: 52 (1). Dead: 1–3, 2–2, 3–1, 5–1, 6–4, 7–4, 8–2. Stn. 120.

Scaphander lignarius (L.)
Living: 105 (1), 113 (1), 127 (1), 150 (1), 152 (1), 159 (1). Dead: 1–2, 3–1. Stn. 120.

Philine quadripartita Ascanius
Living: 1 (1), 4 (13), 12 (2), 32 (1 ?), 44 (1 ?), 49 (1 ?), 51 (3 ?), 52 (4 ?), 53 (2), 71 (1), 119 (6 ?), 141 (11). Dead: 1–1 ?, 2–1 ?, 14–1. Stn. 79.

P. catena (Montagu)
Dead: 113 (1).

Class Scaphopoda

Dentalium entalis L.
Living: 127 (1). Dead: 116 (3), 117 (5), 120 (9), 124 (1), 125 (2), 126 (1), 127 (6).

Dentalium vulgare da Costa
Living: 7 (1), 8 (1), 41 (4), 61 (2), 62 (1), 104 (1), 163 (1). Dead: 5–2, 7–3, 8–5, 9–2, 10–5, 12–5, 13–4, 14–7. Stns. 22, 24, 32, 74–2. A proportion of the dead shells were worn and could not be identified with certainty. However, the records indicate no overlap of *D. vulgare* and *D. entalis*, so that the above records are probably all of *D. vulgare*.

Class Cephalopoda

Sepiola atlantica d'Orbigny 54 (1); 60 (1).

PHYLUM COELENTERATA

Ceriathus lloydi Gosse
45 (*ca.* 50), 46 (2), 47 (2), 48 (13), 90 (3), 91 (1), 103 (2), 104 (3), 107 (1), 112 (7), 116 (3), 120 (1), 153 (11), 163 (4), 165 (1).

PHYLUM ANNELIDA

Aphrodite aculeata L.
1 (1), 4 (7), 11 (1), 12 (1), 20 (2), 48 (1), 52 (1), 57 (1), 67 (2), 69 (1), 71 (6), 72 (1), 73 (4), 74–1 (1), 76 (1), 77 (1), 83 (1), 84 (1), 104 (1).

Chaetopterus variopedatus (Renier)
Living: 134 (C). Empty tubes: 1–4, 2–3, 3–2, 4–2, 5–1, 13–1. Stn. 120.

Arenicola marina (L.)
66 (2 ?), 73 (2).

Pallasia murata Allen
5 (1), 102 (1), 116 (1). Empty tubes: 117 (1).

PHYLUM ECHIUROIDEA

Maxmulleria (= *Thalassema*) *lankesteri* (Herdman)
18 (*ca.* 6), 77 (*ca.* 6), 79 (1), 80 (*ca.* 20).

PHYLUM SIPUNCULOIDEA

Golfingia (= *Phascolosoma*) *elongata* (Keferstein)
11 (8), 22 (*ca.* 15).

G. (= *Phascolosoma*) *vulgaris* (Blainville)
5 (2), 11 (1), 28 (1), 29 (1), 47 (3), 48 (1), 77 (1 ?), 97 (1 ?), 103 (2), 108 (2), 109 (2 ?), 117 (1), 139 (1).

Golfingia (= *Phascolosoma*) sp.
124 (1), 163 (1).

Phascolion strombi (Montagu)
1 (3), 4 (96), 12 (3), 19 (29), 20 (1), 42 (3), 45 (1), 50 (2), 52 (20), 53 (2), 61 (1), 127 (1), 153 (1), 157 (1), 163 (6).

Phascolosoma (= *Physcosoma*) *granulatum* (F. S. Leuckart)
93 (1 ?), 95 (1 ?).

Sipunculus nudus L.
127 (1).

SUB-PHYLUM CRUSTACEA

Squilla desmaresti Risso
22 (1), 57 (1), 102 (1).

Pseudione callianassae Kossmann
Gill-parasite of *Callianassa*, see Pike (1953). Female with attached male, forming 1 pair. 43 (1 pr), 107 (1 pr), 109 (2 pr), 152 (1 pr).

Ione thoracica (Montagu)
Gill-parasite of *Callianassa*. See Pike (1953). 44 (1 pr), 108 (♀), 109 (1 pr), 173 (♀).

Gyge branchialis Cornalia & Panceri
Gill-parasite of *Upogebia*. 5 (1 pr, on *U. stellata*), 108 (1 pr, on *U. deltaura*).

Alpheus glaber (Olivi)
101 (1), 152 (2 ?).

Jaxea nocturna (Chiereghin)
47 (1), 152 (2).

Callianassa subterranea (Montagu)
42 (4), 43 (1), 44 (*ca.* 13), 45 (*ca.* 4), 48 (1 ?), 103 (1), 104 (1), 105 (1), 106 (1), 107 (17), 108 (19), 109 (26), 110 (*ca.* 20), 112 (2), 117 (1), 124 (1), 127 (1), 152 (12), 171 (3), 172 (11), 173 (11).

Upogebia deltaura (Leach)
1 (1 ?), 5 (1), 11 (1), 17 (1), 18 (4), 25 (1), 40 (3), 47 (2), 81 (1), 90 (1 ?), 99 (2), 102 (7), 103 (3), 104 (1), 108 (3), 112 (5), 113 (1), 116 (15), 117 (7), 119 (2), 139 (1), 153 (1).

U. stellata (Montagu)
5 (1), 7 (1), 104 (1), 116 (1), 139 (5), 148 (1).

Corystes cassivelaunus (Pennant)
50 (1), 51 (1), 66 (1), 69 (1), 71 (1), 75 (1), 141 (2), 159 (1), 172 (1).

Atelecyclus septemdentatus (Montagu)
101 (1), 111 (1), 116 (1), 126 (1).

Thia polita Leach
105 (1).

Goneplax angulata (Pennant)
5 (2), 49 (1), 51 (1), 52 (1), 108 (1).

PHYLUM ECHINODERMATA

Astropecten irregularis (Pennant)
43 (3), 44 (1), 45 (2), 49 (1), 105 (1), 106 (2), 127 (1), 136 (1), 140 (1), 141 (6), 150 (1), 151 (1), 153 (1), 159 (1).

Asterina gibbosa (Pennant)
121 (1).

Henricia sanguinolenta (O. F. Müller)
100 (3), 101 (4), 128 (1).

Asterias rubens L.
16 (1), 76 (1), 115 (6), 116 (1), 118 (1), 120 (1), 127 (1), 130 (1), 133 (4), 138 (1), 140 (1), 141 (5), 163 (2), 167 (5), 169 (1), 171 (3).

Marthasterias glacialis (L).
128 (2), 130 (3), 138 (1), 155 (2), 156 (1).

Ophiothrix fragilis (Abildgaard)
25 (1), 82 (5), 84 (5), 86 (40), 87 (2), 98 (2), 115 (2), 116 (1), 120 (1), 123 (124), 124 (7), 125 (1), 126 (1), 128 (3), 130 (1), 133 (7), 134 (2), 137 (1), 140 (1), 141 (2), 142 (740), 149 (1), 152 (1), 158 (2).

Ophiocomina nigra (Abildgaard)
115 (7), 116 (6), 117 (1), 123 (5), 125 (1), 127 (5), 137 (1), 140 (11), 142 (41), 148 (4), 149 (2).

Amphiura chiajei Forbes
134 (3), 155 (1).

A. securigera (Düben & Koren)
155 (1), 159 (1).

A. filiformis (O. F. Müller)
1 (6), 4 (1), 19 (3), 20 (2), 32 (61), 41 (16), 42 (15), 43 (8), 44 (4), 45 (11), 48 (1), 49 (15), 50 (3), 51 (18), 52 (14), 53 (20), 105 (2), 106 (5), 107 (4), 108 (3), 109 (1), 110 (9), 111 (10), 112 (1), 116 (9), 117 (1), 119 (120), 124 (6), 126 (19), 127 (5), 148 (2), 153 (3), 154 (1), 160 (7), 163 (1), 166 (1), 169 (5), 170 (9), 171 (9), 172 (38), 173 (1).

Acrocnida brachiata (Montagu)
4 (1 ?), 12 (36), 13 (1), 14 (2), 19 (8), 20 (2), 32 (1), 41 (1), 42 (1), 50 (22), 51 (1), 60 (1), 63 (10), 66 (3), 67 (5), 71 (1), 72 (1), 73 (1), 74–1 (2), 76 (1), 77 (2), 79 (9), 83 (1), 105 (1), 116 (1 ?), 119 (1), 124 (273), 125 (1), 126 (21), 127 (24), 141 (2), 169 (1).

Ophiura texturata Lamarck
4 (1), 19 (1), 41 (1), 52 (1), 66 (1), 67 (10), 68 (25), 69 (19), 70 (12), 71 (17), 72 (22), 73 (1), 79 (4), 81 (2), 82 (1), 83 (1), 110 (2), 111 (2), 112 (2), 113 (1), 116 (1), 134 (1), 140 (1), 141 (1), 152 (1), 159 (9), 160 (9), 163 (2), 166 (2).

O. albida Forbes
5 (1), 19 (1), 45 (1), 52 (9 ?), 56 (1), 60 (2), 71 (2), 72 (6), 73 (1), 74–2 (1), 75 (2), 76 (1), 79 (7), 81 (2), 82 (5), 83 (27), 84 (1), 87 (27), 88 (3), 90 (1), 91 (7), 112 (3), 118 (3), 122 (2), 123 (3), 127 (2), 134 (1), 148 (1), 155 (1), 163 (4).

O. affinis Lütken
105 (1), 141 (1), 157 (1), 165 (1), 171 (2).

Ophiura sp.
53 (3).

Echinus sp.
Mainly small specimens, of either *E. esculentus* L. or *Psammechinus miliaris* (Gmelin). 39 (1), 48 (1), 55 (1), 56 (2), 57 (1), 85 (4), 86 (1), 93 (1), 97 (1), 100 (1), 101 (1), 102 (1), 128 (1*), 154 (1). (* *E. esculentus*.)

Echinocyamus pusillus (O. F. Müller)
48 (1), 49 (1), 82 (2), 83 (3), 91 (2), 104 (7), 120 (1), 134 (1), 137 (1), 139 (1), 142 (1), 154 (1), 155 (1), 171 (1).

Spatangus purpureus O. F. Müller
87 (2), 88 (8), 135 (2), 157 (1).

Echinocardium cordatum (Pennant)
42 (4), 43 (20), 44 (*ca.* 19), 45 (9), 46 (2), 49 (2), 50 (10), 51 (5), 52 (1 ?), 53 (4), 60 (*ca.* 15), 63 (1 ?), 65–1 (*ca.* 16), 65–2 (10), 66 (3), 67 (15), 68 (*ca.* 34), 69 (3), 70 (1), 71 (*ca.* 36), 72 (3), 73 (10), 74–1 (2), 76 (5), 82 (9), 88 (1), 105 (*ca.* 8), 106 (20), 107 (1), 108 (1), 110 (5), 111 (1), 119 (5), 124 (5), 126 (3), 127 (5), 136 (2), 141 (2), 150 (2), 151 (*ca.* 6), 159 (1), 160 (10), 169 (*ca.* 11), 170 (6), 171 (2), 172 (2), 173 (5).

E. flavescens (O. F. Müller)
129 (3).

E. pennatifidum Norman
113 (1).

Echinocardium sp.
123 (1), 139 (1), 140 (1), 164 (1).

Holothuria forskali Delle Chiaje
122 (1 ?), 130 (1), 152 (1).

Cucumaria elongata Düben & Koren
107 (1), 152 (2), 153 (1), 173 (1).

C. normani Pace
142 (1), 167 (2).

Thyone sp.
81 (1 ?), 117 (4), 134 (2 ?), 137 (1 ?), 139 (1 ?), 148 (2 ?), 150 (1).

Pseudocucumis mixta Östergren
149 (1), 154 (1), 155 (1).

Holothuroidea. Genus not determined.
112 (1), 113 (1), 115 (1), 116 (8), 134 (2), 135 (2).

Leptosynapta inhaerens (O. F. Müller)
4 (1), 43 (1), 45 (2), 47 (1), 107 (1), 108 (1), 109 (1), 110 (*ca.* 5), 124 (3), 127 (1), 152 (2), 160 (1), 171 (1), 172 (2), 173 (3).

Labidoplax digitata (Montagu)
50 (*ca.* 3), 52 (1), 53 (3), 104 (1), 117 (1), 119 (2), 120 (2), 124 (1), 126 (1), 127 (4), 129 (1), 136 (2), 140 (3), 141 (14), 159 (1), 160 (1).

Synaptidae (*Leptosynapta* or *Labidoplax*)
42 (1), 51 (1).

PHYLUM CHORDATA

Amphioxus lanceolatus (Pallas)
135 (1), 137 (6).

Cepola rubescens L.
108 (1).

LIST OF LAMELLIBRANCHS TAKEN LIVING, BUT NOT INCLUDED IN THE ABOVE FAUNA LIST

Most occurred at only 1 or 2 stations

Arca lactea L., *Modiolus barbatus* (L.), *Modiolus* sp., *Musculus marmoratus* (Forbes), *Musculus* sp., Anomiidae spp., *Lima loscombi* Sowerby, *Cardium ovale* Sowerby, *C. scabrum* Philippi, *C. exiguum* Gmelin, *C.* (*Parvicardium*) sp., *Hiatella* spp., *Thracia distorta* (Montagu).

RECORDS OF WESTERN SPECIES OTHER THAN IN DREDGE SURVEY

(See Fig. 6)

Great West Bay. Sixty stations worked by Mr E. Ford in November 1923, using a $\frac{1}{10}$ m^2 Petersen grab. One haul at most stations. Much the same area was covered in a survey in 1948 (Holme, 1950).

Plymouth area. Ford's (1923) survey. Also twenty stations worked in 1950 (Holme, 1953), and a few other grab stations, all outside Plymouth breakwater.

South Cornish coast. Nine stations in St Austell Bay, November 1950, and thirty-eight stations in June and November 1954, from St Austell Bay to the Lizard. One or two grab hauls at most stations. Also shore-collecting records at St Antony-in-Roseland, Helford and Manaccan in the Falmouth area, 1949–60.

Records, apart from those published as above:

Chlamys tigerina. Nil.

Myrtea spinifera

Station M 1	50° 16·1′ N.,	4° 46·0′ W.	18 m	16.xi.50 One
Station M 2	50° 16·35′ N.,	4° 46·2′ W.	18 m	16.xi.50 One

Gouldia minima

Station S 29	50° 05·9′ N.,	5°03·8′ W.	20 m	5.xi.54	Two

(Still taken in small numbers, living, in the Eddystone shell-gravel).

Gari tellinella

Station M 7	50° 19·9′ N.,	4° 43·8′ W.	10 m	16.xi.50	One
Station S 11	50° 10·65′ N.,	4° 57·1′ W.	27 m	3.xi.54	One
Station S 24	49° 59·09′ N.,	5° 09·5′ W.	18 m	4.xi.54	One
Station S 25	49° 58·6′ N.,	5° 09·6′ W.	35 m	4.xi.54	One
Station S 27	50° 01·5′ N.,	5° 05·3′ W.	13 m	4.xi.54	One

Tellina pygmaea

Station S 17	Helford River.		9 m	4.xi.54	Seven

Also record from Plymouth Sound (Marine Biological Association, 1957).

Thracia villosiuscula

Station S 19	Helford River.		15 m	4.xi.54	Five
Station S 23	49° 59·95′ N.,	5° 08·75′ W.	27 m	4.xi.54	One
Station S 26	49° 58·85′ N.,	5° 07·2′ W.	51 m	4.xi.54	One

Cochlodesma praetenue. See Holme (1953) for several Plymouth records

Station S 9	50° 11·35′ N.,	4° 57·15′ W.	22 m	3.xi.54	Fourteen
Station S 11	50° 10·65′ N.,	4° 57·1′ W.	27 m	3.xi.54	Thirteen
Station S 17	Helford River.		9 m	4.xi.54	One

St Antony-in-Roseland (shore)—2 specimens, 1955

Dentalium entalis. Not taken at any of the stations detailed above.
The only additional record, at the mouth of the Channel, is:

Station 24	49° 05′ N.,	6° 35′ W.	*ca.* 120 m.	6.vi.51	Three in 0·45 m²

(also taken subsequently in this area from R.R.S. *Discovery II*). The record of *Dentalium* sp. at Station 31 in Great West Bay (Holme, 1950) is of *D. vulgare.*

STATION LIST

Dates of sampling as follows:

Stations 1– 25: 11–13 November 1958
26– 53: 28–29 January 1959
54–112: 20–27 May 1959
113–142: 15–17 July 1959
148–173: 15–16 December 1959

Stations are listed in order from west to east, the samples making up each of the fourteen areas (Fig. 4) being shown unbracketed.

Abbreviations

DEPOSITS

m., mud; s., sand; gr., gravel; st., stones; r., rock; sh., shell; Lith., *Lithothamnion* gravel; c., coarse; f., fine; m., medium; cln., clean. —, no soil sample. +, interpret as 'with some'.

SIZE OF SAMPLE

¼, ½, ¾, 1, 2, etc., numbers of baths-full (1 bath = roughly 20 l.).
½ F, F, dredge net half-full, full.
T, sample contained a number of stones, no finer soil.
W, soil sample small or nil, due to washing out.
II, two dredge-hauls.

TREATMENT

H, hosed down or otherwise roughly sorted on deck.
2·2, all sample passed through 2·2 mm sieve.
2·2(1)/H, one bath-full passed through 2·2 mm sieve, the rest sorted on deck.

2·2/5/H, a small (unmeasured) portion passed through 2·2 mm sieve, another portion through 5 mm sieve, the rest sorted on deck.

—, no sample.

All, whole sample kept (unsieved).

Sieve meshes (square holes): 2·2, 2·5, 5 and 11·5 mm.

Station	Position		Depth (m)	Deposit	Size of sample	Treatment
Area 1						
121	50° 06·2′ N.	5° 31·55′ W.	18	st.	T	H
124	50° 05·3′ N.	5° 30·65′ W.	29	f.m.s.	1	2·2
126	50° 04·8′ N.	5° 30·5′ W.	37	m.s.	1/3	2·2
122	50° 06·3′ N.	5° 30·3′ W.	20	gr. + st.	½ T	H
127	50° 03·1′ N.	5° 29·85′ W.	48	m.s.	II. W; W	2·2
125	50° 05·0′ N.	5° 29·35′ W.	33	gr. + st.	T	H
123	50° 06·0′ N.	5° 29·0′ W.	26	f.sh.gr. + st.	1½	2·2(1)/H
129	49° 59·85′ N.	5° 28·05′ W.	54	—	W	All
128	50° 02·9′ N.	5° 25·4′ W.	32	st. + gr.	T	H
130	50° 01·2′ N.	5° 22·2′ W.	41	? r.	—	H
(131	50° 00·4′ N.	5° 18·0′ W.	26·5	? r.	—	H)
(132	49° 59·3′ N.	5° 08·8′ W.	26·5	? r.	—	—)
Area 2						
133	49° 59·45′ N.	5° 07·4′ W.	39	gr.	½	H
134	50° 01·55′ N.	5° 04·55′ W.	27	m. + Lith.	1½	2·2(1/10)/11·5
135	50° 01·45′ N.	5° 04·05′ W.	35	f.sh.gr.	2	2·2(1/10)/5/H
136	50° 05·75′ N.	5° 05′ W.	8	cln.s.	2	2·2
138	50° 07·2′ N.	5° 04·3′ W.	?17	Lith.	F	2·2/11·5/H
137	50° 06·65′ N.	5° 03·4′ W.	17	Lith.	F.	2·2/5/11·5/H
139	50° 08·35′ N.	4° 57·5′ W.	32	m. + f.gr.	F	2·2/5/H
140	50° 11·6′ N.	4° 56·25′ W.	25	cln.s.	¾	2·2
141	50° 12·0′ N.	4° 56·3′ W.	17	cln.s.	2½	2·2
142	50° 12·55′ N.	4° 50·2′ W.	27	st.	T	H
(120	50° 13·4′ N.	4° 50·15′ W.	26	c.s.	2	2·2)
Area 3						
117	50° 17·25′ N.	4° 43·55′ W.	29	gr.s. + m.	F	2·2/5/11·5/H
118	50° 16·45′ N.	4° 46·0′ W.	19	st.	T	H
119	50° 17·5′ N.	4° 45·35′ W.	17	f.m.s.	3	2·2
116	50° 18·45′ N.	4° 36·6′ W.	35	m.s. + shale	F	2·2(1)/H
115	50° 18·6′ N.	4° 30·85′ W.	46	—	W	All
(114	50° 19·1′ N.	4° 27·2′ W.	37/35	st.	T II	—)
113	50° 19·1′ N.	4° 22·25′ W.	16	f.sh.gr.	1½	2·2(1)/H
148	50° 19·1′ N.	4° 19·3′ W.	32	st. + m.gr.	1½	H
149	50° 19·7′ N.	4° 15·8′ W.	24	st.	½	H
150	50° 19·75′ N.	4° 15·25′ W.	23	—	W	All
151	50° 19·75′ N.	4° 14·9′ W.	14	cln.f.s.	½	2·2
Area 4						
152	50° 17·8′ N.	4° 16·9′ W.	42	m.	F	2·2(1½)/H
153	50° 17·2′ N.	4° 07′ W.	43	m.s. + gr.	1½	2·2(¾)/H
154	50° 17·0′ N.	4° 03·9′ W.	37·5	sh.gr.	1	2·2(¼)/H
155	50° 16·9′ N.	4° 01·4′ W.	37·5	sh.gr.	1½	2·2(¼)/H
156	50° 16·3′ N.	3° 59·8′ W.	39·5	—	T	All
157	50° 15·05′ N.	3° 58·85′ W.	39·5	cln.sh.gr.	3–4	2·2(¼)/5(1)/H
158	50° 16·5′ N.	3° 56·5′ W.	28·5	m.sh.gr.	3–4	H
(162	50° 14·4′ N.	3° 56·4′ W.	31	—	W	—)
159	50° 15·25′ N.	3° 55·55′ W.	31	f.s.	½	2·2
160	50° 15·8′ N.	3° 53·95′ W.	22	f.s.	¼	2·2
161	50° 15·0′ N.	3° 53·5′ W.	25·5	st. + sh.gr.	F	2·2(¼)/5(¼)/H

Station	Position		Depth (m)	Deposit	Size of sample	Treatment
Area 5						
112	50° 12·1′ N.	3° 29·3′ W.	58	c.s. + sh.	F	2·2(1/10)/H
163	50° 14·1′ N.	3° 38·6′ W.	14·5	st. + sh.	¾	2·2(1/10)/5
164	50° 14·95′ N.	3° 37·4′ W.	9	f.s.	½	2·2
165	50° 15·65′ N.	3° 35·75′ W.	10	c.s.	W	All
166	50° 15·9′ N.	3° 37·5′ W.	15·5	f.s.	¾	2·2
167	50° 15·8′ N.	3° 38·15′ W.	12	shale	T	All
168	50° 16·75′ N.	3° 38·4′ W.	13	m.s. + sh.	F	2·2(1/10)/H
169	50° 17·4′ N.	3° 37·4′ W.	13	f.m.s.	½	2·2
170	50° 18·6′ N.	3° 36·0′ W.	13	f.m.s.	1	2·2
111	50° 16·35′ N.	3° 24·7′ W.	53	—	W	All
Area 6						
171	50° 19·5′ N.	3° 31·15′ W.	39·5	m. + sh.	½	2·2
172	50° 21·7′ N.	3° 29·35′ W.	32	s.m. + sh.	F	2·2
173	50° 20·2′ N.	3° 23·4′ W.	54	f.s.m.	F	2·2(1)/H
51	50° 25·0′ N.	3° 29·75′ W.	11	m.	2	2·2
52	50° 25·8′ N.	3° 29·7′ W.	11	m.	1	2·2
53	50° 26·4′ N.	3° 29·7′ W.	11	m.	1	2·2
49	50° 30·5′ N.	3° 28·6′ W.	12	m.s.	1½	2·2(1)/H
110	50° 20·0′ N.	3° 20.5′ W.	51	f.m.s.	F	2·2(2)/H
109	50° 23·85′ N.	3° 19·4′ W.	45	f.m.s.	F	2·2(2)/H
108	50° 26·65′ N.	3° 14·7′ W.	37	f.m.s.	F	2·2(2)/H
Area 7						
50	50° 31·85′ N.	3° 28·6′ W.	9	m.s.	2	2·2(1)/H
48	50° 31·55′ N.	3° 18·25′ W.	23	c.m.s.	F	5(1)/H
47	50° 33·7′ N.	3° 13·65′ W.	26	m.s. + sh.	F	2·2(1)/H
107	50° 28·6′ N.	3° 09·7′ W.	37	f.m.s.	F	2·2(2)/H
46	50° 35·1′ N.	3° 08·25′ W.	28	s.m.	1	2·2
106	50° 29·85′ N.	3° 03·2′ W.	37	f.m.s.	½	2·2
45	50° 36·05′ N.	3° 02·4′ W.	28	s.m. + sh.	1	2·2
105	50° 30·4′ N.	2° 57·2′ W.	39	m.s.	2	2·2
44	50° 36·4′ N.	2° 56·4′ W.	29	m.s. + sh.	1	2·2
104	50° 30·7′ N.	2° 50·7′ W.	37	c.m.s.	F	2·2(1)/H
Area 8						
43	50° 36·4′ N.	2° 50·2′ W.	31	f.m.s.	½	2·2
103	50° 30·6′ N.	2° 45·35′ W.	37	c.m.s.	F	2·2(1/10)/H
42	50° 35·8′ N.	2° 44·2′ W.	31	s. + gr.	1	2·2
102	50° 30·8′ N.	2° 39·1′ W.	36	c.s. + gr.	F	2·2(1/10)/H
41	50° 34·45′ N.	2° 38·85′ W.	33	m.s. + gr.	F	H
3	50° 37·25′ N.	2° 25·6′ W.	11	m.gr.	3–4	5(2)/H
2	50° 37·3′ N.	2° 25·4′ W.	10	m.gr.	½ F	5(2)/H
1	50° 37·1′ N.	2° 24·6′ W.	15	m.	1	2·2
4	50° 36·95′ N.	2° 22·9′ W.	18	m.s.	II. 2; 2	2·2
5	50° 37·0′ N.	2° 20·6′ W.	18	m.gr.	F	H
Area 9						
7	50° 36·4′ N.	2° 19·75′ W.	19	m.gr.	3	5(2)/H
101	50° 29·6′ N.	2° 15·5′ W.	32	st.	¾ F (T)	H
8	50° 34·6′ N.	2° 14·45′ W.	27	gr. + st.	2	H
9	50° 33·95′ N.	2° 11.2′ W.	30	sh.gr. + st.	2–3	H
10	50° 34·0′ N.	2° 08·7′ W.	26	sh.gr.	2	H
100	50° 30·4′ N.	2° 06·4′ W.	37	st. + gr.	¾ F (T)	H
99	50° 31·1′ N.	1° 57·5′ W.	36	st. + c.s.	F (T)	H
(28	50° 37·9′ N.	1° 55·3′ W.	11	cln.sh.gr.	F	H)
(29	50° 37·5′ N.	1° 55·15′ W.	15	m.gr.	F	H)
(27	50° 37·7′ N.	1° 54·9′ W.	13	f.gr. + m.	F	H)
(33	50° 39·0′ N.	1° 54·7′ W.	11	cln.s.	1/3 F	H)

Station	Position		Depth (m)	Deposit	Size of sample	Treatment
Area 9 (*cont.*)						
(34	50° 39·3′ N.	1° 54·5′ W.	11	cln.s.	?	H)
(35	50° 39·7′ N.	1° 54·5′ W.	11	gr. + s.	?	H)
17	50° 39·65′ N.	1° 54·2′ W.	13	m.gr.	3–4	5(1)/H
(40	50° 37·7′ N.	1° 54·2′ W.	16	f.m.gr.	F	H)
(31	50° 40·25′ N.	1° 54·15′ W.	8	m. + st.	?	H)
(39	50° 37·0′ N.	1° 54·1′ W.	21	s. + gr. + st.	?	H)
(30	50° 39·75′ N.	1° 53·9′ W.	11	m. + gr. + st.	?	H)
*25	50° 37·65′ N.	1° 53·7′ W.	21	m.gr.	F	H
(26	50° 37·65′ N.	1° 53·7′ W.	17	m.gr.	F	H)
(36	50° 38·95′ N.	1° 53·2′ W.	15	m.s. + gr.	?	H)
11	50° 39·65′ N.	1° 53·1′ W.	16	m.	2–3	5(2)/H
(37	50° 37·9′ N.	1° 53·05′ W.	17	s. + gr.	½ F	H)
(38	50° 37·1′ N.	1° 53·0′ W.	23	s.gr. + st.	F	H)
Area 10						
18	50° 41·2′ N.	1° 52·0′ W.	15	m.	F	2·2(2)/H
(32	50° 41·1′ N.	1° 51·9′ W.	15	black m.	F	2·2(2)/H)
*(24	50° 38·6′ N.	1° 49·8′ W.	19	st.	T	H)
12	50° 41·6′ N.	1° 49·3′ W.	13	black m.	II: ¼; ¼	2·2
19	50° 41·75′ N.	1° 47·95′ W.	15	silty s.	II: 1; 1/8	2·2
20	50° 41·5′ N.	1° 46·0′ W.	18	m.s.	1	2·2
13	50° 39·55′ N.	1° 45·7′ W.	10	cln.s.	W	All
(21	50° 41·25′ N.	1° 44·45′ W.	18	m.gr.	F	5 (½)/Rest lost)
(23	50° 39·8′ N.	1° 44·4′ W.	16	? r.	—	—)
14	50° 39·35′ N.	1° 44·3′ W.	17	? cln.s.	W	All
(22	50° 40·7′ N.	1° 43·3′ W.	22	m. + st.	II: 3–4/3–4	5(2)/H)
15	50° 38·65′ N.	1° 39·1′ W.	22	gr. + st.	T	H
16	50° 38·9′ N.	1° 39·9′ W.	22	m. gr.	F	H
98	50° 31·45′ N.	1° 48·6′ W.	34	gr. + st. + c.s.	F	H
97	50° 31·1′ N.	1° 38·7′ W.	33	gr. + st. + c.s.	F	H
Area 11						
96	50° 29·3′ N.	1° 29·4′ W.	33	gr. + st. + c.s.	½ F	H
95	50° 28·7′ N.	1° 18·7′ W.	32	gr. + st.	F (T)	H
(94	50° 30·1′ N.	1° 09·2′ W.	33	chalk	T	—)
93	50° 30·7′ N.	0° 59·6′ W.	27	gr. + st. + c.s.	F	H
92	50° 32·1′ N.	0° 51·1′ W.	22	st. + cln.s.	1	H
54	50° 39·4′ N.	0° 46·9′ W.	16	m.s. + st.	F	H
55	50° 36·5′ N.	0° 38·6′ W.	30	m.s. + st.	2/3 F	H
91	50° 34·7′ N.	0° 35·7′ W.	49	m.c.s. + gr.	F	2·2(1/10)/H
56	50° 37·2′ N.	0° 35·5′ W.	32	m.s. + gr.	F	H
57	50° 38·4′ N.	0° 29·6′ W.	25	st. + m.s.	F	H
90	50° 36·6′ N.	0° 26·4′ W.	37	m.s. + gr. + st.	F	2·2(1/10)/H
Area 12						
58	50° 41·2′ N.	0° 24·6′ W.	20	m.s. + st.	½ F	H
59	50° 42·4′ N.	0° 18·6′ W.	19	m.s. + st.	¼ F	H
89	50° 36·8′ N.	0° 16·9′ W.	54	s. + sh.gr.	F	2·2(1/10)/H
60	50° 44·0′ N.	0° 13·0′ W.	18	cln.s. + sh.	1½	2·2
88	50° 37·7′ N.	0° 07·7′ W.	45	sh. + st.	1/3	H
61	50° 44·7′ N.	0° 06·5′ W.	19	cln.s. + sh.	F	2·2(1)/H
62	50° 45·3′ N.	0° 00·6′ W.	19	m.s.	2	2·2(1)/H
87	50° 37·9′ N.	0° 01·7′ E.	49	st. + little s.	? T	H
63	50° 44·4′ N.	0° 06·0′ E.	21	m.s.	2	2·2(1)/H
86	50° 38·8′ N.	0° 11·1′ E.	32	st.	T	H
Area 13						
64	50° 43·7′ N.	0° 11·5′ E.	21	m. + st.	F	H
85	50° 39·7′ N.	0° 21·1′ E.	31	gr. + st.	T	H

* Conventional fine-mesh dredge.

Station	Position		Depth (m)	Deposit	Size of sample	Treatment
Area 13 (*cont.*)						
84	50° 40·5′ N.	0° 29·9′ E.	34	c.s. + st.	F	H
65	50° 44·6′ N.	0° 31·9′ E.	18	s. + gr.	II W; ¼ F	2·2, H
74	50° 45·1′ N.	0° 37·4′ E.	29/27	gr. + sh.	II W; W	2·2, H
72	50° 47·6′ N.	0° 39·7′ E.	14	c.s.	¾	2·2
73	50° 46·7′ N.	0° 37·8′ E.	18	c.m.s.	¾	2·2
83	50° 41·5′ N.	0° 39·6′ E.	52	c.s. + gr.	½ F	2·2(1)/H
71	50° 48·8′ N.	0° 41·5′ E.	12	s.	1¾	2·2
75	50° 47·1′ N.	0° 43·7′ E.	31	s. + sh. + gr.	2	11·5(1)/H
Area 14						
70	50° 50·1′ N.	0° 43·2′ E.	10	m.s.	½	2·2
69	50° 51·3′ N.	0° 45·0′ E.	10	m.s.	½	2·2
68	50° 52·4′ N.	0° 46·6′ E.	10	m.s.	½	2·2
67	50° 53·3′ N.	0° 48·3′ E.	9	m.s.	½ (W)	2·2
76	50° 49·8′ N.	0° 48·6′ E.	31	m.s.	F	2·2(1)/H
81	50° 46·1′ N.	0° 49·2′ E.	34	c.s. + gr. + st.	F	2·2(¼)/H
82	50° 41·7′ N.	0° 49·4′ E.	40	s. + st.	F	2·2(1)/H
66	50° 54·4′ N.	0° 50·1′ E.	8	m.s.	¾	2·2
77	50° 52·2′ N.	0° 53·2′ E.	27	m.	F	2·5(1)/H
78	50° 57·6′ N.	1° 02·4′ E.	27	m.s.	F	2·5(¾)/H
(79	51° 00·2′ N.	1° 04·2′ E.	18	m.	F	2·2(1)/H)
(80	51° 02·6′ N.	1° 06·6′ E.	17	m.	F	*2·2(1)/H)

* Part of sample lost, only *Maxmulleria* remaining.

REFERENCES USED IN COMPILING TABLES 1–8

Geographical distribution

MOLLUSCA: Allen (1954*a*, *Nucula*); Allen (1954*b*, *Pandora*); Bucquoy, Dautzenberg & Dollfus (1882–98); Forbes & Hanley (1853); Jeffreys (1862–9); Knudsen (1949); Madsen (1949); Thorson (1941).

ECHINODERMATA: Einarsson (1948); Mortensen (1927).

CRUSTACEA: Bouvier (1940); Gustafson (1934).

Distribution around British Isles

Devon and Cornwall: Clark (1906); Ford (1923, and unpublished records from Great West Bay); Professor L. A. Harvey (unpublished fauna list of Scilly Isles); Holme (1950, 1953); Marine Biological Association (1957); Todd (1903, 1906); Vevers (1952). Also some of my own unpublished observations.

French coast: Dautzenberg & Durouchoux (1913–14); Prenant (1932, 1939); Station Biologique de Roscoff (1951). Also my own unpublished observations on the Brittany coasts.

Ireland: Nichols (1900).

Isle of Man: Jones (1956); Moore (1937).

Clyde: Knight (1901); Scottish Marine Biological Association (1961); Todd (1901).

Orkney: Rendall (1956).

North Sea: Birkett (unpublished); Birkett (1953); Davis (1923, 1925); Hagmeier (1925); McIntyre (1958); Stephen (1933, 1934); Ursin (1952, 1960).

Feeding Habits

Allen (1958*a*, Lucinacea); Allen (1958*b*, *Cochlodesma*); Hunt (1925); MacGinitie (1949, *Callianassa* and *Upogebia*); Morton (1959, *Dentalium*); Roushdy and Hansen (1960, *Ophiocomina*); Yonge (1946, *Turritella*).

RE-IDENTIFICATION OF *SPISULA ELLIPTICA*

Ford (1923)

Grab station 35. Five pairs, labelled *S. elliptica*. Rather small shells which appeared to be *S. elliptica*, but which might be *S. solida*.
Grab station 47. Five pairs, labelled *S. elliptica*. Re-identified as *S. solida*.
Conical dredge, 24 August. Two pairs, labelled *S. elliptica*. Re-identified as *S. solida*.
Originally determined by R. Winckworth.

Atkins (1937)

Shells kindly shown me by the late Dr D. Atkins which were used for her work on ciliary mechanisms. Locality: Plymouth area. Labelled *S. elliptica* and determined by R. Winckworth. These were re-identified as *S. solida* (3 pairs), *S. elliptica* (2 valves, not a pair). This explains the differences in ciliation found in two specimens by Dr Atkins. Other specimens collected at the same time were mostly *S. solida*, and a few *S. elliptica*.

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ON THE BIOLOGY OF *CALANUS FINMARCHICUS*

XII. THE PHOSPHORUS CYCLE: EXCRETION, EGG PRODUCTION, AUTOLYSIS*

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With an Addendum

THE TURNOVER OF PHOSPHORUS BY *CALANUS FINMARCHICUS*

By R. J. Conover
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(Text-figs. 1–5)

The return of plant nutrients to sea water by the zooplankton has been investigated by Cooper (1935), Gardiner (1937), and Harris (1959). The first two showed experimentally that living zooplankton organisms rapidly increased the concentration of phosphorus in the sea water, and Harris showed that excretion was important in supplying nitrogen to the phytoplankton of Long Island Sound. The breakdown of dead organisms was studied by Hoffmann (1956) and Krause (1959). Marshall & Orr (1955) made some measurements of the excretion of P by *Calanus finmarchicus* and showed that it was most rapid during the first week after a period of feeding. The very high losses after 2–3 weeks of starvation led them to suppose that the ^{32}P they were measuring had not been in equilibrium through the animals.

In view of the importance of phosphate regeneration as a factor in the productivity of the sea, it was decided to follow in more detail, using the copepod *Calanus*, the role of the zooplankton in the turnover of P.

METHODS

Early experiments on the excretion of soluble P, after feeding with labelled food cultures (Marshall & Orr, 1955), were unsatisfactory because the P excreted disappeared from solution, presumably being taken up by bacteria on the walls of the container. The loss of P from the body was therefore measured instead.

* Contribution no. 1050 from the Woods Hole Oceanographic Institution.

Addition of antibiotics to the water, however, prevents the loss by bacterial action, and from 1958 the excretion was measured only in the water in which the *Calanus* were kept. Streptomycin and chloromycetin were the antibiotics used (Oppenheimer, 1955), but it was later found that the chloromycetin had a depressing effect on feeding. In the 1959–60 experiments penicillin and streptomycin were used, neither of which had a deleterious effect in a concentration of 50 mg/l.

The P in the body was measured only at the beginning and end of an experiment. Initial measurement was made by putting the *Calanus* in a Perspex cell of the same diameter as a planchette (2 cm) and having, excavated in the centre, a cylindrical cavity 5·5 mm in diameter and 1·5 mm deep. The *Calanus* is put in the cavity in a drop of water and the excess sucked off until the water is just level with the top of the cell. For comparable readings the *Calanus* must be measured always in the same position. The highest readings are got if the dorsal side is uppermost because of the dorsal position of the ovary, the most highly radioactive tissue. The reading when the ventral side is up is the lowest and that when the copepod is on its side is intermediate. The last position was chosen because it is the easiest to get a *Calanus* to adopt. The cavity was not large enough to let them move freely, but an occasional one may have turned over during the course of the reading. Since the cell is appreciably higher than the planchettes normally used and since water is present, the relation between wet and dry readings had to be found by reading a considerable number of *Calanus*, first alive in the cell and then torn and dried on a planchette. The factor varied considerably from about 1 to 1·25, and the average used was 1·185. To avoid damaging the *Calanus* used for an experiment, only one 40 sec initial reading was taken.

Another improvement in accuracy was made by using a liquid counter of 10 ml. capacity for the water samples instead of a series of dried 0·2 ml. samples. Conversion factors for the liquid counters were made against readings on planchettes and the mean of a large number of counts taken. Considering the variability of these two conversion factors and the short duration of the initial reading of the *Calanus* it is satisfactory that the initial direct reading and the final calculated reading (made by adding the radioactivity gained by the water to the reading of the *Calanus* body at the close of the experiment) usually agreed fairly well (see, for example, Table 2). The calculated initial value is used in the tables and figures.

Calanus of the stage desired were picked out of a tow-netting and put in a culture of radioactive phytoplankton, usually either *Skeletonema costatum* or *Syracosphaera elongata*. Either *Calanus finmarchicus* or *C. helgolandicus* were used according to their abundance. If necessary they were changed to fresh culture at intervals and when they had been fed for a given time they were washed individually and their radioactivity measured in the Perspex cell. They were then put singly in calibrated bottles of about 35 ml. capacity

filled with membrane-filtered water containing antibiotics. The bottles were put in dark cloth bags and kept in a tank at 10° C.

Earlier experiments had shown that, although a *Calanus* can take up ^{32}P from solution, the amount is negligible when compared with that taken up in the food.

In some experiments half of the copepods were starved and half fed by the addition of non-radioactive culture to the membrane-filtered water. Survival was good, but on several occasions the starved *Calanus* survived better than the fed (see Table 1). To measure excretion each *Calanus* was transferred to a fresh bottle and duplicate samples from the original bottle read in a liquid counter. Measurements were usually made after 6 and 24 h. and thereafter at longer intervals.

At the end of an experiment the *Calanus* bodies were either torn up and dried on a planchette or else a final reading of the living animal was made in a Perspex cell. Occasionally the body was torn and dried on a coverslip laid on a planchette and was subsequently used for total P analysis.

Most excretion experiments were done on females because they feed best, and in the winter because they are not then laying eggs. Several, however, were done on laying females and, before sampling, the water in each bottle was examined for eggs. When these were present they were washed and dried and their radioactivity measured. A few drops of a 1 % solution of ethylene-diamine-tetracetic acid was added per 100 ml. of medium in these experiments to ensure the healthy development of the eggs (Cleland, 1953).

On some occasions analyses of total phosphorus were made by Harvey's method (1948). From 1 to 6 *Calanus* were used in each determination.

EXCRETION

The results of the excretion experiments are shown in Tables 1–4 and Figs. 1–3. Table 2 and Fig. 2 give one experiment set out in full to show the extent of the individual variation and the comparison of the initial and final readings of the bodies. On the whole it can be said that the shorter the period of feeding on radioactive culture, the more rapid is the initial excretion of phosphorus. This can be said only of the average curves; in each series there may be individuals which excrete more quickly or more slowly than the rest. The results of the longer term feeding were not so plain. Of the two series fed for 7 days, one excreted during the first few days at a slower rate than the other which was only slightly slower than the 1-day feeding series. Of two series fed for 2 weeks or longer one was more like the slower and one like the faster of the 7-day feeding series. In a final experiment where the *Calanus* were fed first on radioactive food for a week, then on non-radioactive food for a week, the resulting curve was the same as the slow 7-day series. It seems probable that in the experiments showing a very gradual fall, the ^{32}P had reached equilibrium throughout the body. In the curves showing a rapid

TABLE 1. EXCRETION OF *CALANUS* AFTER FEEDING FOR DIFFERENT TIMES

f, *C. finmarchicus*; h, *C. helgolandicus*

Date ...	9–22.xii.59		15–28.xii.59		4–16.xi.59		24.xi.–8.xii.59		17.xi.–1.xii.59		1–15.xii.59		10–26.v.58		28.xii.59–12.i.60		1–7.vi.60	20–27.iv.60
^{32}P feeding ...	3 h		3 h		1 day		1 day		7 days		7 days		14 days		18 days		7 days then non-r.a. 7 days	2 days
No. and stage	7♀h	6♀h	7♀h	7♀h	7♀h	6♀h	7♀h	7♀h	7♀h	7♀h	7♀h	7♀h	5♀f	5♀f	7♀h	7♀h	15♀f	14V
Treatment	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Starved	Starved
Time	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss	Av. % loss
6 h	18·6	9·9	15·7	9·7	21·0	9·6	15·5	7·1	6·6	7·1	5·6	3·8	6·3†	5·4†	5·9	5·5	1·8	—
1 day	36·4	18·5	30·1	18·8	34·9	23·6	35·4	21·1	13·3	18·2	11·5	8·4	—	—	12·7	13·4	7·5	15·2
2 days	—	—	—	—	—	—	—	—	—	—	—	—	13·1	11·7	—	—	13·1	24·8
3 days	52·4	41·4	44·1	39·3	49·9	42·1	49·4	42·5	22·8	32·2	23·9	17·5	$16·1_4$	$17·6_4$	23·1	27·6	—	30·1
4 days	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	21·4	—
5 days	$57·4_6$	49·9	—	—	58·4	49·9	—	—	—	—	—	—	$23·6_4$	$24·3_4$	—	—	—	37·4
6 days	—	—	55·1	52·8	—	—	59·5	55·4	38·9	46·7	29·7	28·7	—	—	$37·8_6$	42·5	27·6	—
7 days	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	43·8
8 days	$63·7_6$	55·8	—	—	62·9	59·0	—	—	—	—	—	—	—	—	—	—	—	—
9 days	—	—	All dead	62·1	—	—	66·8 All dead	63·2	52·5	57·0	—	—	—	—	$47·9_4$	53·8	—	—
10 days	—	—	—	—	—	—	—	—	—	—	$43·4_1$	$43·5_6$	—	—	—	—	—	—
11 days	—	—	—	—	—	—	—	—	—	—	—	—	$39·0_4$	$42·5_4$	—	—	—	—
12 days	—	—	—	—	67·6	68·1	—	—	—	—	—	—	—	—	$56·6_4$	$64·0_6$	—	—
13 days	$73·3_2$	66·9	—	70·3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14 days	—	—	—	—	—	—	—	$73·1_5$	$68·1_6$	$68·8_3$	$54·3_1$	$55·1_6$	—	—	—	—	—	—
15 days	—	—	—	—	—	—	—	—	—	—	—	—	—	—	$61·7_4$ all dead	$72·1_5$	—	—
16 days	—	—	—	—	—	—	—	—	—	—	—	—	$51·1_4$	$54·7_4$	—	—	—	—

Suffix indicates number of *Calanus*. † 12 hours

TABLE 2. EXCRETION OF FEMALE *CALANUS* FED 3 HOURS WITH ^{32}P LABELLED FOOD

All counts, No./ml./min.

Fed ...	1		2		3		4		5		6		7		Average % loss	
Days	Count	% loss	Count	% loss	Count	% loss	Count	% loss	Count	% loss	Count	% loss	Count	% loss		Sum
0 (wet)	45,990		20,880		26,340		39,040		37,260		39,860		26,070			
0 (calc.)	45,334		20,511		25,231		36,250		35,500		38,955		23,133			
¼ (6 h)	11,510	25·4	4,399	21·4	4,096	16·2	5,637	15·5	6,191	17·4	6,023	15·5	4,306	18·6	18·6	
1 (18 h)	8,389	18·5	4,521	22·0	3,945	15·6	4,180	11·5	6,965	19·6	6,128	15·7	4,963	21·4	17·8	36·4
3	5,957	13·1	4,707	23·0	2,900	11·5	6,483	17·9	5,833	16·4	5,236	13·4	3,828	16.5	16·0	52·4
5	3,232	7·1	327	1·6	2,039	8·1	Killed	—	1,744	4·9	1,349	3·5	1,119	4·8	5·0	57·4
8	2,505	5·5	1,350	6·6	1,367	5·4	—	—	2,228	6·3	3,338	8·6	1,235	5·3	6·3	63·7
13	6,686	14·7	3,563	17·4	2,645	10·5	—	—	3,038	8·6	5,641	14·5	6,064	26·2	9·6	73·3
		Dead		Dead								Dead		Dead		
Body at end	7,055		1,644		8,239		19,950		9,501		11,240		1,618			

Starved ...	1		2		3		4		5		6		Average % loss	
Days	Count	% loss	Count	% loss	Count	% loss	Count	% loss	Count	% loss	Count	% loss		Sum
0 (wet)	32,010		65,120		33,670		49,870		31,660		32,000			
0 (calc.)	35,764		71,035		36,073		48,107		32,211		32,134			
¼ (6 h)	7,820	21·9	4,757	6·7	3,329	9·2	4,064	8·4	2,067	6·4	2,096	6·5	9·9	
1 (18 h)	2,905	8·1	3,918	5·5	2,696	7·5	2,188	4·6	3,292	10·2	5,036	15·7	8·6	18·5
3	5,801	16·2	16,070	22·6	10,120	28·0	11,980	24·9	6,094	21·4	7,838	24·4	22·9	41·4
5	1,927	5·4	8,506	12·0	4,851	13·4	5,365	11·1	1,945	6·0	956	3·0	8·5	49·9
8	1,257	3·5	4,943	7·0	3,178	8·8	3,434	7·1	2,043	6·3	767	2·4	5·9	55·8
13	2,524	7·1	9,541	13·4	4,635	12·9	6,756	14·0	4,150	12·9	1,981	6·2	11·1	66·9
Body at end	13,530		23,300		7,264		14,320		11,810		13,460			

TABLE 3. EFFECT OF TEMPERATURE ON EXCRETION

Fed one day and starved during experiment

Date ...	7–21. i. 60			27. i–8. ii. 60		
Calanus ...	7 ♀ f	7 ♀ f	7 ♀ f	7 ♀ f	7 ♀ f	5 ♀ f
Temp. (° C)	5°	10°	15°	5°	10°	15°
	Mean loss %	Mean loss %	Mean loss %	Mean loss %	Mean loss %	Mean loss %
6 h	1·7	2·2	3·9	6·7	4·1	6·7
1 day	6·2	7·9	10·4	15·8	13·1	15·9
3 days	18·1	20·1	27·1	27·1	30·6	35·0
6 days	—	—	—	40·6	46·4	51·1
7 days	34·0	37·3	48·6	—	—	—
9 days	—	—	—	51·5	55·9	60·4
11 days	44·5	46·9	61·9*	—	—	—
12 days	—	—	—	59·1	63·6	67·3†
14 days	50·6	52·9	69·2*	—	—	—

* Average of 6. † Average of 4. f = *finmarchicus*

TABLE 4. EXCRETION OF LAYING FEMALE *CALANUS*

Mean loss %

Date ...	4–9. v. 58				4–9. v. 58			
^{32}P feeding ...	1 day				1 day			
Calanus ...	5 *C. finmarchicus* (starved)				5 *C. finmarchicus* (fed)			
	Excretion				Excretion			
	Eggs	Total	Egg layers	Non layers	Eggs	Total	Egg layers	Non layers
8 h	0	7·2	—	7·2	—	18·3	—	18·3
1 day	$6·1_2$	13·6	23·9	6·7	$5·5_1$	8·6	11·9	8·6
2 days	$1·0_1$	13·9(3)	11·1	15·3	0	18·5(4)	—	18·5
3 days	—	—	—	—	—	—	—	—
4 days	—	—	—	—	—	—	—	—
5 days	$3·1_1$	10·3(3)	10·0	10·4	0	9·2(4)	—	9·2

Date ...	5–11. iv. 60				6–12. vii. 60			
^{32}P feeding ...	1 day				5 days, then non-^{32}P 4 days			
Calanus ...	14 *C. finmarchicus* (starved)				7 *C. finmarchicus* (fed)			
	Excretion				Excretion			
	Eggs	Total	Egg layers	Non layers	Eggs	Total	Egg layers	Non layers
8 h	—	—	—	—	—	—	—	—
1 day	$6·1_6$	12·3	18·1	7·9	$0·4_1$	6·8	3·9	7·4
2 days	$12·1_1$	14·6	15·9	10·1	$0·2_2$	4·5	3·2	5·0
3 days	$2·2_6$	10·4	13·5	8·2	$2·1_3$	5·5(6)	2·9	8·1
4 days	0	4·9	—	4·9	$2·0_3$	2·2(5)	2·2	2·2
5 days	$1·3_1$	2·6(2)	2·4	2·7	$0·7_2$	1·6(5)	0·9	2·2
6 days	0	3·5(2)	—	3·5	$1·5_3$	3·0(5)	2·3	3·9
7 days	0	2·7(2)	—	2·7	—	—	—	—

Suffix indicates number of *Calanus* laying; number in brackets indicates number averaged.

initial fall, on the other hand, a large proportion of the ^{32}P must be in a labile form. This is confirmed by the results of the total P analyses (see p. 477).

There is, during the first day or two, a difference in excretion of ^{32}P between fed and starved *Calanus*. Those being fed (on non-radioactive food) excrete more than those being starved (see Fig. 2 and Tables 1 and 2). The more food taken in therefore the more is metabolized and this agrees with the

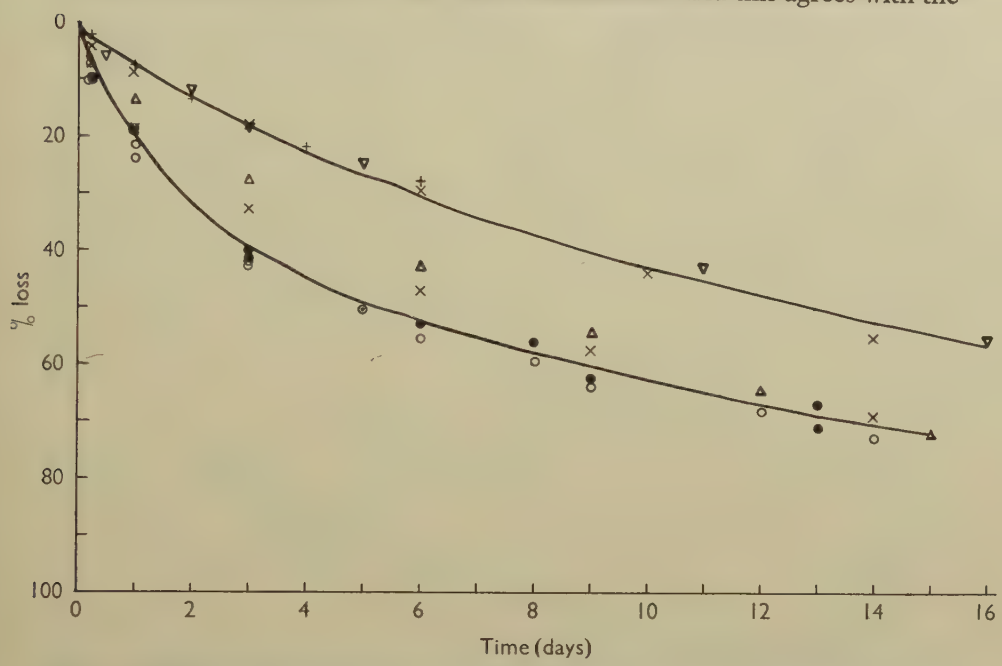

Fig. 1. Effect of short-term (lower curve) and long-term (upper curve) feeding on excretion in starved female *Calanus*. ●, 3 h; ○, 1 day; ×, 7 days; ▽, 14 days; △, 18 days; + 7 days radioactive, then 7 days non-radioactive.

fact that the percentage of food digested is high, even in *Calanus* that are kept in a rich culture and are producing faecal pellets rapidly.

Two experiments were made on the effect of temperature on excretion in starved *Calanus* (Table 3 and Fig. 3). The temperatures compared were 5°, 10°, and 15° C. Although there was the usual variation and the curves for individuals in the three series usually overlapped, the averages show a slightly greater excretion as the temperature rises. One peculiarity of these particular experiments was that in all the *Calanus*, excretion, although following the usual curve, was much lower than normal. Most of the excretion experiments were done in late autumn and winter on *C. helgolandicus*, a few females of which are to be found throughout that time. The temperature experiments were done, however, with *C. finmarchicus* females newly moulted from over-wintering Stage V. They must have been immature and low in total P (see

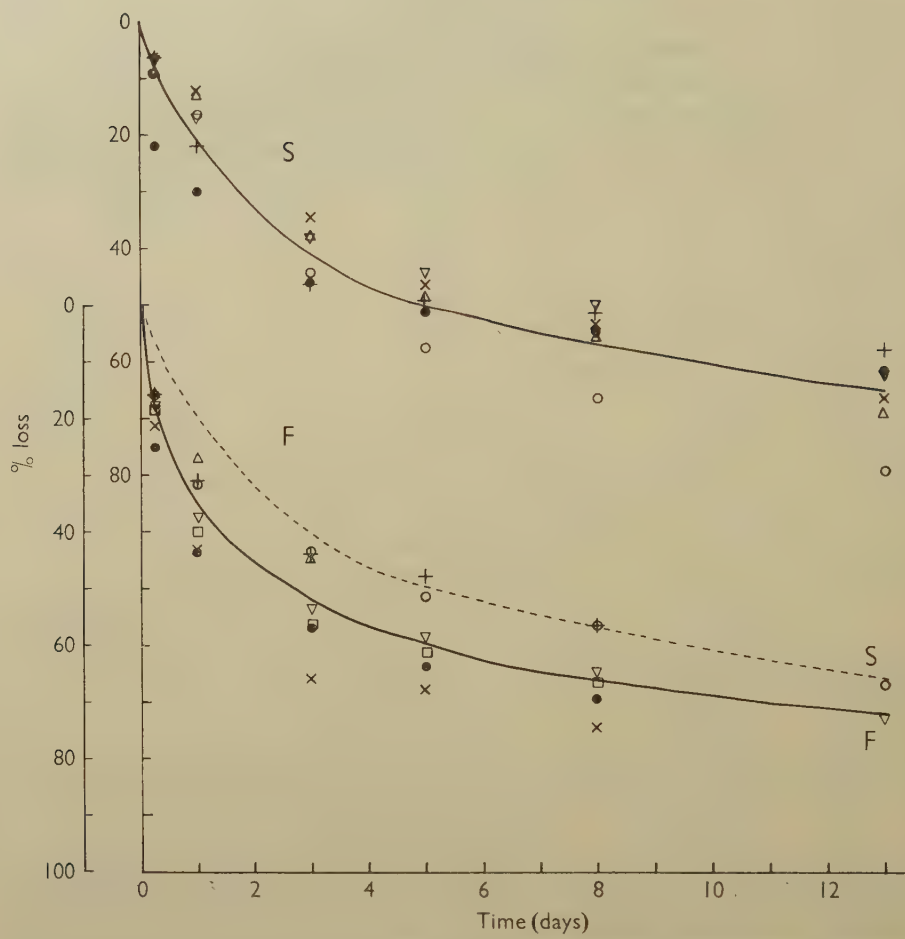

Fig. 2. Excretion in fed and starved *Calanus* after 3 h feeding with labelled culture to show variation between individuals. The curve for starved is repeated (dotted line) for comparison with that for fed. Each symbol represents one *Calanus* in both fed and starved.

p. 478). The *C. finmarchicus* females were not tested again until April when they were ripe and may have belonged to the next generation. The excretion figures had then risen and were much the same as the *C. helgolandicus* in autumn.

Stage V were used only once, in April, and their average excretion was slightly lower than that of females fed for the same length of time.

EGG PRODUCTION

It has previously been found (Marshall & Orr, 1955), by rough dissection of *Calanus* females fed on cultures labelled with ^{32}P, that a large proportion of this is concentrated in the ovary and reproductive system. Some experiments

were therefore made to find out how large a proportion of the ^{32}P entering the *Calanus* body could be recovered in the eggs laid.

Preliminary experiments showed that *Calanus* females fed on radioactive and non-radioactive food laid much the same number of eggs, i.e. the ^{32}P in the concentrations used did not in itself affect the ovary.

When ripe female *Calanus* are brought into the laboratory almost all of them lay eggs on the first night. If fed they go on laying at irregular intervals for some weeks or months. For most experiments ripe females were picked

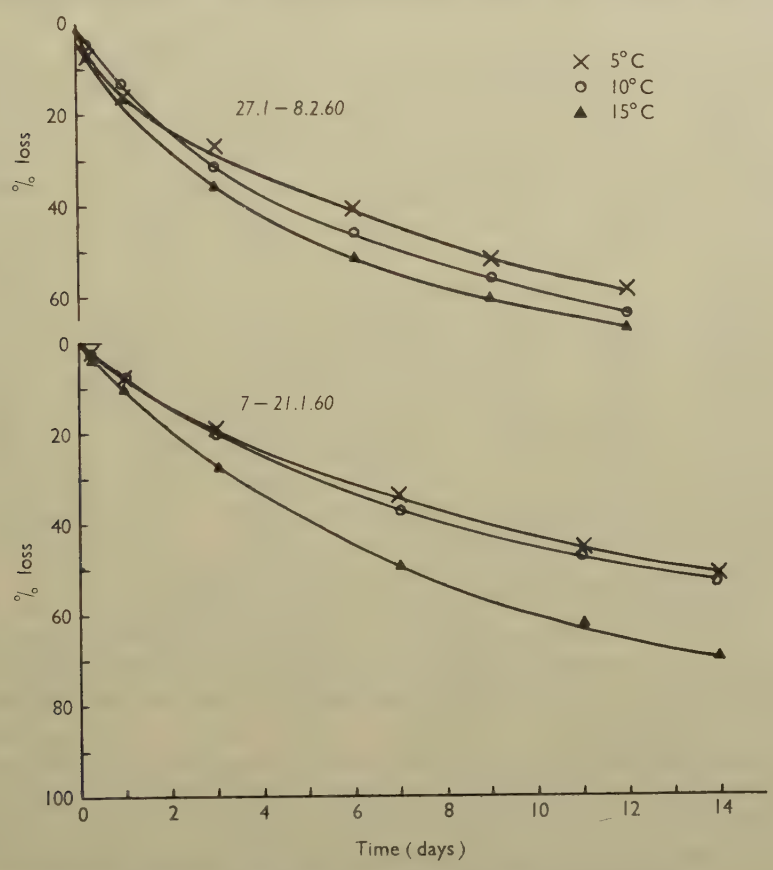

Fig. 3. Effect of temperature on excretion in starved female *Calanus*.

out from a tow-netting and put overnight into a culture of radioactive phytoplankton. Next morning they were washed and the activity measured in a Perspex cell. The eggs laid were also washed and read dry. These two figures added give the initial body value, although it will be slightly too low owing to the overnight excretion.

The results varied according to the time of year at which the experiments were carried out. In an experiment made in August 1959, the 24 females used survived badly, but those which lived more than 5 or 6 days produced eggs which contained 15–34 % of the initial ^{32}P. These *Calanus* may have

TABLE 5. EGG LAYING EXPERIMENTS IN FEBRUARY

Date	No.	Time (days)	Total no. eggs laid	% ^{32}P in eggs	% ^{32}P in body at end	% ^{32}P excreted (excluding eggs)
C. helgolandicus						
3–29. ii. 60	1	22	111	33·8	6·7	89·9
3–29. ii. 60	2	22	142	26·3	5·2	92·9
4–24. ii. 60	3	21	59	27·8	12·1	83·3
4–24. ii. 60	4	21	58	24·8	10·2	86·4
4–24. ii. 60	5	21	72	25·1	13·1	82·5
4–24. ii. 60	6	21	48	17·0	10·1	87·8
5–24. ii. 60	7	20	49	21·1	13·0	83·5
6–24. ii. 60	8	19	55	14·2	18·1	78·9
7–24. ii. 60	9	18	152	31·1	10·1	85·3
C. finmarchicus						
9–27. ii. 60	10	19	93	10·4	11·2	87·5
10–27. ii. 60	11	18	192	48·7	7·0	86·4
10–27. ii. 60	12	18	169	52·0	15·2	68·3
10–27. ii. 60	13	18	153	44·3	12·5	77·5
10–27. ii. 60	14	18	83	21·2	18·3	76·8
10–27. ii. 60	15	18	82	24·7	12·1	83·9
10–27. ii. 60	16	18	139	28·2	13·2	81·6
10–27. ii. 60	17	18	173	37·0	13·6	78·4
11–27. ii. 60	18	17	139	44·6	20·1	63·7
11–27. ii. 60	19	17	92	40·4	11·4	80·9
11–27. ii. 60	20	17	96	37·0	15·6	75·2

been laying for some time in the sea, and later experiments were carried out in early spring either before, or just about, the time at which *Calanus* eggs normally appear here in the plankton in numbers. All the females were given less than 24 h of radioactive feeding and were then fed on non-radioactive culture for a varying time (Table 5).

At this time the sea was poor in phytoplankton and the eggs in the females were developing slowly. In many the number of eggs per lay rose rather than fell towards the end of the period, but in spite of this the radioactivity per lay decreased steadily. The maximum percentage of ^{32}P produced in any one lay was 25–30 %, and this was invariably in the first. The results from two of these individuals are shown in Figs. 4, nos. 11 and 16, and a summary of the results is given in Table 5. Because of the rapid loss of ^{32}P later experiments were stopped after about a week.

In the two final series (Table 6) the *Calanus* were caught about 6 a.m., which is shortly after the time (midnight to 4 a.m.) at which the majority of females lay in spring, and were fed on radioactive culture till evening. They

were then removed, washed, measured for ^{32}P and given non-radioactive food. In the experiment on 7 March only two *Calanus* out of 24 had laid eggs by evening, so that, apart from these, the readings of the live bodies gave the true figures for the initial values. Of the 25 *Calanus* females set up in the same way on 14 March, 10 had laid eggs by evening. These two experiments were carried on for only 6 and 5 days. The results are given in Table 6, and Fig. 4, nos. 21, 23, 24, but only for these *Calanus* which laid more than once and survived till the end of the experiment.

It is surprising how large a proportion (up to 38 %) of the ^{32}P ingested can come out again within 24 h as eggs. The largest number of eggs is usually laid on the first night and the highest percentage return of ^{32}P accordingly appears then also. There is usually a steady decrease thereafter but occasionally, with a larger number of eggs laid, the downward trend is temporarily reversed. At the end of the experiments the *Calanus* were killed and the ^{32}P remaining in the body measured. When this, plus the value of the eggs laid, is subtracted from the original reading, the difference may be taken as the phosphorus lost by excretion.

In the two experiments where the *Calanus* were caught early and fed during the day the total percentage returned as eggs in the 6 days following the radioactive meal averaged 47·3 %; the maximum was 72·4 %. These high figures indicate an insistent demand for phosphorus on the part of the ovary and explain why *Calanus* females lay freely only when well fed.

It seemed possible that the ^{32}P might be adsorbed on the surface of the egg and not really incorporated in its substance. To test this, some ripe *Calanus* females were fed on labelled phytoplankton and their eggs collected and washed. Half were then measured for ^{32}P; half were allowed to hatch and the ^{32}P in the resulting nauplii measured. There was no significant difference between the radioactivity of eggs and nauplii from the same lay, which indicates that the ^{32}P was not merely on the surface.

In Fig. 4 the loss of ^{32}P as eggs is plotted cumulatively, but the loss in excretion is calculated only by difference and the curve for this drawn to resemble the usual excretion curves. Fig. 4, nos. 11, 16, show the results of the experiments in February. In the March experiments (Fig. 4, nos. 21, 23, 24, 25) the ripe females obviously have a large number of eggs ready, or almost ready to lay, and these were produced during the next 5 or 6 days. It seems clear from these two series of experiments that when much of the P is used in egg production, the excretion is reduced. Even when we subtract from the initial reading the P in the eggs, the excretion, as a percentage of the remainder (Tables 5 and 6, last column), is still low for those which lay well.

Three series of experiments were made on laying females in which excretion of ^{32}P was measured as well as that produced in eggs (Table 4). In the first two of these, the females were given labelled food for one day only and starved for the rest of the experiment. The average loss by excretion followed

TABLE 6. EGG LAYING EXPERIMENTS IN MARCH

Date	No.	Number of, and percentage of ^{32}P in, eggs laid on day: Day of capture		1		2		3		4		5		6		Total no. eggs laid	% ^{32}P in eggs	% ^{32}P in body at end	% ^{32}P excreted (excluding eggs)
7–13.iii.60	21	58	17·0	—	—	15	5·2	46	10·1	5	0·9	6	0·8	15	1·9	145	36·0	19·1	70·2
	22	—	—	71	35·0	43	14·8	33	7·2	24	3·8	10	1·4	—	—	181	62·1	22·1	41·7
	23	—	—	56	37·8	38	16·9	30	8·2	28	5·4	14	2·2	13	1·8	179	72·4	20·0	27·5
	24	—	—	65	33·7	—	—	37	9·2	—	—	27	3·9	—	—	129	46·7	34·1	36·0
	25	—	—	—	—	128	37·0	47	12·8	—	—	36	5·0	41	5·3	252	60·0	28·6	28·5
14–19.iii.60	26	—	—	24	13·3	64	16·2	10	1·8	17	2·1	—	—	—	—	115	33·4	21·4	67·9
	27	47	36·9	—	—	29	12·1	20	3·9	12	1·7	4	0·4	—	—	112	55·1	25·3	43·6
	28	32	22·6	—	—	32	8·7	14	2·8	26	4·4	32	3·3	—	—	136	41·8	25·6	56·0
	29	—	—	35	22·3	62	18·4	30	6·3	—	—	—	—	—	—	127	47·0	23·4	55·9
	30	—	—	49	28·9	32	12·3	21	4·1	—	—	—	—	—	—	102	45·3	34·4	37·1
	31	—	—	27	22·8	26	9·7	8	1·9	16	3·1	—	—	—	—	77	37·5	38·8	37·9
	32	45	18·6	—	—	65	21·5	31	5·1	—	—	—	—	—	—	141	45·2	19·0	65·3
	33	—	—	17	0·3	—	—	—	—	22	4·6	—	—	—	—	39	4·9	33·5	64·8
	34	—	—	—	—	20	11·6	53	22·3	—	—	9	1·2	—	—	82	35·1	26·8	58·7

closely the curve appropriate for one day's feeding, but when the excretion by egg-layers and non-egg-layers on any one day was compared, the former were found to have excreted considerably more than the latter. In the third series the females were fed 5 days on radioactive and then 4 days on non-radioactive food before measurements started, so that the ^{32}P would be in equilibrium

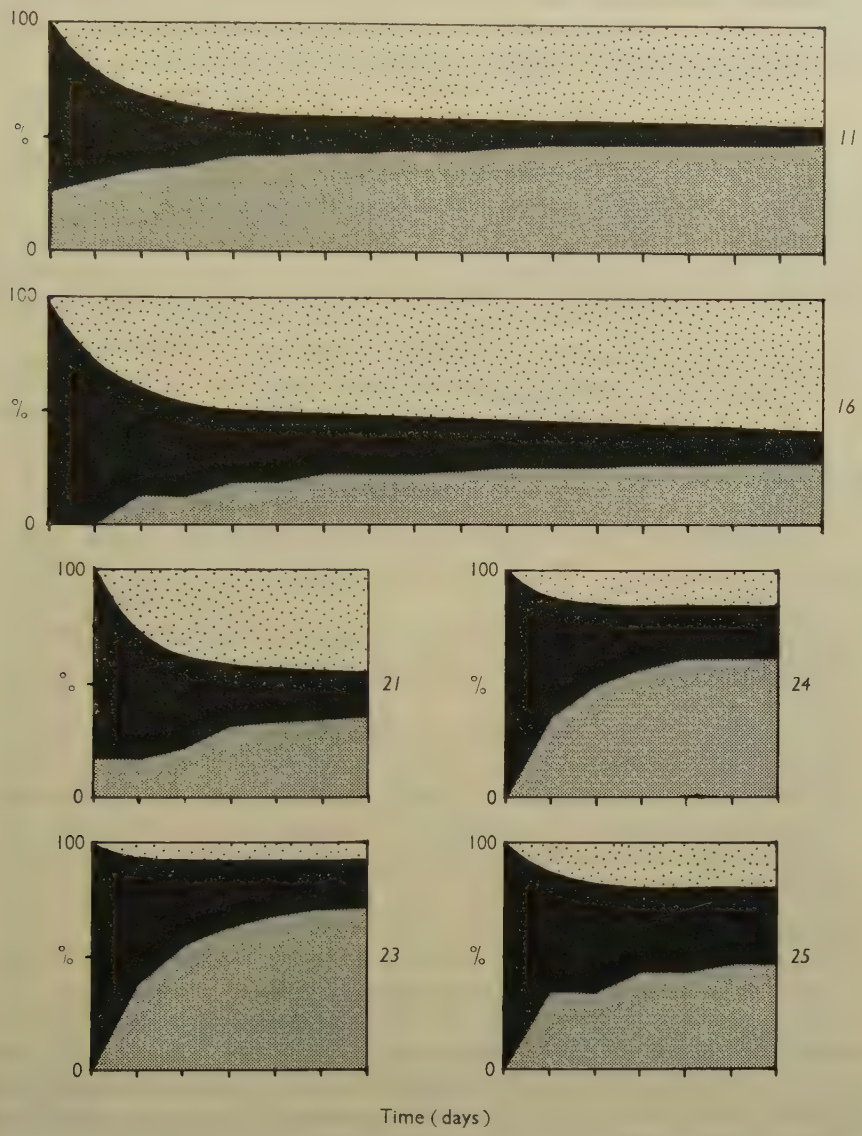

Fig. 4. Fate of one meal in laying female *Calanus finmarchicus*. Black, ^{32}P in body; dark stippling, in eggs; light stippling, in excretion.

throughout the body. Non-radioactive feeding was continued throughout the experiment, and some of the females laid regularly although the number of eggs was small. Excretion of ^{32}P was low, and there was no marked difference between egg-layers and non-egg-layers.

A possible explanation of this difference is that in the starved females the P for excretion must come from the pool of labelled P in the body, whereas in the fed it will come mainly from the food newly taken in which was not labelled and would therefore not be measured.

TOTAL PHOSPHORUS IN *CALANUS*

It was unexpected that the losses in excretion as measured by ^{32}P should be so high and some total P analyses were made for comparison.

Those at Woods Hole were done through the kindness of Dr Bostwick H. Ketchum. In these several *Calanus* from the same batch as the experimental animals were used to give initial P values, but there was so much variation

TABLE 7. PHOSPHORUS ANALYSES

Experiment	Initial		Final		% change total P	% change ^{32}P
	No. *Calanus**	μg P/*C*.	No. *Calanus**	μg P/*C*.		
March 1960						
Fed 6 days	15 ♀ (3)	1·39	10 ♀ (2)	1·60	+15	—
Fed 7 days			2 ♀ (1)	1·95	+40	—
Starved 6 days			10 ♀ (2)	0·93	−33	—
Starved 7 days			3 ♀ (1)	0·84	−40	—
April 1960						
Fed 3 days	16 ♀ (4)	1·95	8 ♀ (2)	1·43	−27	—
Starved 2 days			6 ♀ (2)	0·87	−55	—
Fed 7 days			10 ♀ (2)	1·80	−8	—
Starved 6 days			6 ♀ (2)	1·01	−49	—
Starved 4 days			11 ♀ (4)	1·14	−42	−62
April 1960						
Starved 2 days	12 ♀ (3)	1·80	9 ♀ (3)	1·43	−21	−40
Starved 4 days			11 ♀ (3)	1·32	−27	−44
June 1960						
Starved 6 days	16 ♀ (4)	1·33	13 ♀ (3)	0·97	−31	−29
April 1960						
Starved 7 days	10 V (2)	1·18	7 V (2)	0·84	−30	−45
May 1960						
Fed 1 day	20 V (4)	1·80	20 V (4)	1·68	−7	—
Fed 2 days			20 V (4)	1·36	−25	—
May 1960						
Autolysed 3 days	Estimated	1·62	9 ♀ (2)	0·015	−91	−94

* Number of determinations in brackets

between individuals that this cannot be a very reliable figure. In the later analyses at Millport a considerable number of controls (up to 20 in groups of 3–5) gave a more reliable initial result. The mean of these was compared with the mean of groups of 1–5 at the end of the experiment. The results are shown in Table 7.

There is evidently a considerable variation in the P content of individual *Calanus*. The values vary from 1·12 to 2·39 μg/*Calanus* female. They are partly dependent on the state of maturity and may also be dependent on feeding either before or after capture. Even after feeding in the laboratory, however, the total P was increased only in two experiments, and it was more usual for there to be a slight fall. At Woods Hole the chloromycetin used was later found to inhibit feeding in *Calanus* to a marked extent, and the loss in fed *Calanus* there was greater than at Millport where penicillin was used instead. After starving, the loss in total P was always considerable.

At Woods Hole the total P was measured in individual *Calanus* at the end of the experiment and was compared with the average initial value. In spite of the great individual variation, the average percentage change given by both the radioactive and the total P methods agreed well for starved *Calanus* in long- as well as in short-term feeding experiments. Even in one *Calanus* which had lost as much as 70 % the agreement was close. For fed *Calanus* the average values by the two methods naturally differed more.

At Millport most of the total P analyses were done after short-term feeding experiments, and in these the loss calculated from radioactive measurements was much above that from total P. In the one long-term feeding experiment (1. vi. 60) the results from the two methods were closer.

Stage V was used in two experiments. The initial values were only slightly below those for females and were 1·18–1·80 μg P/*Calanus*. As with females, feeding in the laboratory did not prevent the loss of total P and in a short-term feeding experiment excretion as measured by ^{32}P was much above that as measured by total P.

It appears that it is only when the radioactive feeding period is long enough for the ^{32}P to be in equilibrium throughout the body that the two methods give comparable results.

Since such a high proportion of the P goes to the ovary it seemed that it should be possible to measure the total P in *Calanus* eggs. In April eggs were collected from 50–60 females of both *C. finmarchicus* and *C. helgolandicus*. The numbers were 2227 and 2452 respectively. In duplicate estimations the first contained 0·68 and 0·71 and the second 0·96 and 1·08 μg P/100 eggs. In May these analyses were repeated after the females had been fed overnight to find out if this would raise the P content of the eggs. The numbers were 878 and 815, the P content 0·65 and 0·65 μg P/100 eggs for *C. finmarchicus* and 1·08 and 1·12 for *C. helgolandicus*. It thus appears that short-term feeding does not increase the P content of the individual egg laid. Since at the peak of the egg-laying period as many as 60 to 100 eggs may be produced in a single lay, a *Calanus* can part with up to a third of its total P in one lay. This confirms the egg-laying experiments with ^{32}P.

One would also expect unripe females to be less rich in P than ripe, and this was tested in June 1960. Most females in the tow-nettings were ripe, but

a few in an early stage of reproductive development were obtainable. The analyses showed that the unripe contained 1·27 and the ripe 2·01 μg P/*Calanus*.

Stage V may contain as much P as do females, depending presumably on their state of nutrition. The early copepodite stages rarely have more than a small quantity of fat, but Stage V varies from having only a small amount to having an enormous bolster which fills most of the body. In the rough dissections already mentioned of *Calanus* fed with labelled P, we found that much was in the fat (up to 40 %). Since after moulting into females the fat rapidly disappears with the growth of the eggs, it was assumed that the fat was used to build up the eggs. Total P was estimated in Stage V of different fatness and found to vary from 0·71 μg/*Calanus* in lean up to 1·84 in fat. In samples taken in mid-July most of the fat Stage V were larger than the lean (length of cephalosome 2·32 and 2·02 mm) and perhaps belonged to a different generation. In a comparison of 5 lean and 5 fat of the same length, however, the P content was 0·79 and 1·24 μg P/*Calanus*.

LIBERATION OF PHOSPHORUS FROM DEAD *CALANUS*

Phosphorus is returned to the sea by the break-down of dead zooplankton. To find out the rate at which this happens, some experiments were made by measuring the loss of ^{32}P from dead *Calanus*. After feeding overnight with cultures labelled with ^{32}P, the radioactivity of the body was measured in a Perspex cell and the animals killed and put singly in bottles of membrane-filtered sea water. To some of these the usual antibiotics were added, to some antibiotics plus toluol, to others only chloroform, and finally there were some controls with no addition. The bottles were kept rotating slowly on a vertical wheel to keep the bodies moving and so simulate sinking in the sea. They were kept at a temperature of about 18° C.

In some experiments (Table 8) the *Calanus* were killed by gentle heat. A surprising feature was the high rate of loss of ^{32}P even after only 24 h. After 3 days it was over 90 % and analyses of total P confirmed this. Even in 2 h, the loss, though variable, might be as high as 75 %. The results were much the same in the controls and in those to which chloroform had been added.

Since the heating might have reduced enzyme or bacterial activity some *Calanus* were killed by freezing, but the results confirmed those in which the *Calanus* had been killed by heat.

The breakdown of P compounds may be caused by autolysis or by bacterial action since the presence of antibiotics, toluol or chloroform does not entirely rule out the latter. From the point of view of re-cycling of P in the sea, it is perhaps immaterial which is responsible but since all the *Calanus*, including the controls, released P at much the same rate, autolysis seems the most likely explanation.

TABLE 8. AUTOLYSIS OF DEAD *CALANUS* AT 18° C

(Loss %)

Date ...	11–14.v.60		17–19.v.60		4–6.vii.60						24–26.v.60			
Method of killing ...	Heat		Heat		Heat		Heat		Heat		Cold		Cold	
Addition ...	P and S		P and S		P and S		$CHCl_3$		None		P and S		P and S and toluol	
No. *Calanus* ...	9♀		9♀		3V 1♀		4V 1♀		4V		8♀		8♀	
Loss %	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
2 h	—	—	4·6–74·6	31·8	*43·0–70·3	60·1	*64·4–73·4	70·2	*45·1–66·3	57·1	—	—	68·9–82·6	75·8
8 h	—	—	21·9–83·5	55·4	—	—	—	—	—	—	†73·0–86·4	80·4	75·4–88·1	82·0
1 day	70·1–90·4	81·0	53·7–92·8	74·4	76·9–83·1	80·4	78·3–83·9	81·5	68·2–96·3	79·1	88·0–94·0	92·2	88·3–95·2	91·8
2 days	88·3–95·2	90·8	67·6–94·9	81·4	82·2–89·3	85·6	85·3–89·8	87·3	74·0–97·0	84·1	93·0–97·0	95·6	93·1–98·0	95·5
3 days	92·0–96·1	93·7	—	—	—	—	—	—	—	—	—	—	—	—

P = penicillin; S = streptomycin; * 5 h; † 5–7 h.

DISCUSSION

One of the most important points to consider in any experiment using ^{32}P is the relation between the radioactive and the total P. The total P analyses done in conjunction with the excretion experiments show that not all the P compounds in the *Calanus* body are labelled immediately. It needs a period of feeding of at least a week before labelled P is in equilibrium throughout the body. Some compounds are more labile and in them P is metabolized more rapidly than in others. From the rate of loss of metabolized P by excretion in *Calanus* which have been fed long enough for the ^{32}P to be in equilibrium (Table 1, p. 466) we can estimate that the biological half-life is about 14 days. From this we can calculate the turnover time, i.e. the time required to liberate the amount of P present in a *Calanus*. This is given (Zilversmit, Entenman & Fishler, 1943) as half-life × 1·44, i.e. about 20 days.

The large reduction in P which can be tolerated during starvation was unexpected, especially as it did not seem to affect the activity of the animals. It was usual, however, to find a high mortality when the ^{32}P loss rose to about 70 %.

The high rate of excretion during the first 24 h after a short-term feeding experiment with labelled culture has a bearing on the use of such cultures in the measurement of filtering rates. In the early excretion experiments (Marshall & Orr, 1955) the *Calanus* were fed for a week, excretion on the first day was 10 % or less, and it was concluded that the effect in feeding experiments would be negligible. *Calanus* in a feeding experiment lasting usually about 18–24 h is, however, more properly compared with those in excretion experiments after one day's feeding and the 'volume filtered', at least that part of it which is calculated on food assimilated, may have thus been underestimated by an amount varying from 10 % up to 50 %.

The figures we gave for volumes filtered varied from less than 1 ml. up to 84 ml./24 h. A corrected maximum figure of 120 ml./day is above the average found by Gauld (1951) when calculating filtration by cell counts (70 ml.) but is still much below that postulated by some recent workers (Cushing, 1959; Steele, 1959) to account for changes in the phytoplankton populations in the sea.

A surprising feature of the results of total P analyses is the relatively big difference in the P content of individual *Calanus*. Apart from this the value probably varies seasonally. There is usually more P in females than in Stage V, more in ripe than in unripe females, and more in fat than in lean Stage V. The normal value for the P content of one female may be taken as 0·05–0·06 μg atom P (1·55–1·86 μg P) which corresponds to the mean value for 100 ml. sea water at E 1 in the English Channel in winter (Armstrong & Butler, 1960).

Our earlier observations showed that in females much of the ingested P was

rapidly transferred to the ovary and one of the most striking facts about the P metabolism is the large quantity which goes into egg production. Barnes & Barnes (1956) also noted this in the freshwater copepod *Cyclops strenuus*. In this species females without egg-sacs contained two-thirds of the P of females with them, i.e. as in *Calanus* the P in the eggs was about one-half the P in the rest of the body. In *Cyclops* there was 0·35 μg P/100 eggs, in *Calanus finmarchicus* 0·68 μg in eggs of about the same size.

The observations on the fate of the ^{32}P of one short feeding period (p. 473) show that in the course of 5–6 days up to 70 % may appear as eggs. The percentage of P excreted in these actively laying females is lower than usual (Fig. 4) and this seems to indicate that egg-laying females use their food more efficiently.

Richman (1958) has made a detailed study of the energy budget of *Daphnia pulex*, and has found that in the pre-adult stage 55–58 % of the energy assimilated was used for growth. Once reproduction had begun only 3–4 % was used for growth and 52–70 % went into the eggs and young. His experiments dealt with animals over the greater part of their life span, whereas ours deal with a relatively short feeding time, but they confirm one another in indicating that the metabolism of these Crustacea is efficiently geared for reproduction.

These studies on excretion and autolysis emphasize the importance of the role of zooplankton in the regeneration of P in the sea. Some of the P is inorganic and some organic (Cooper, 1935; Hoffmann, 1956). The organically bound P may be available for plant growth either directly (Harvey, 1945; Chu, 1946) or after further breakdown.

Harris (1959) has already shown that excretion by the zooplankton is important in the re-cycling of nitrogen in Long Island Sound. In spring and summer there, zooplankton excretion accounts for half the nitrogen required by the phytoplankton.

Calanus in a rich food culture can produce faecal pellets at the rate of one every 5 or 6 min. These are, however, low in P (Harvey, Cooper, Lebour & Russell, 1935) and these authors thought that this was because P from undigested cells was liberated directly into the water as phospho-proteins and phospholipids. Our digestion experiments and the present results, however, suggest that assimilation of P is high; the rate of loss from faecal pellets is low.

The seasonal and diurnal migrations of the zooplankton and the length of their stay in the surface waters rich in phytoplankton will be significant in deciding whether the regenerated P will be immediately available for plant production or only after its introduction there by vertical mixing.

Hoffmann (1956) measured the rate of breakdown of P in both phyto- and zooplankton after death and his figures for the autolysis of mixed copepod plankton are in fairly close agreement with ours for female *Calanus*. According to him 20–25 % of inorganic P and 30–40 % of organic P is liberated very

shortly after death and between 80 and 90 % in 24 h. We found that breakdown was almost complete in 2 days. It is probably autolytic, and we agree with Hoffmann that bacterial action (Harris, 1959) or enzymes in the water (Kreps, 1934; Steiner, 1938) are of relatively small importance.

Gardiner (1933) measured the rate of sinking of anaesthetized *Calanus* and found it to be dependent on size. For females it was about 15 m/h so that a dead *Calanus* might liberate as much as 75 % of its P in sinking through the top 50 m. It is probable that, after the formation of a thermocline, the growth of the phytoplankton depends more on the excretion of the zooplankton than on nutrients supplied from below or from bacterial decomposition.

We should like to thank the Director and staff of the Woods Hole Oceanographic Institution for their generous support, especially Dr G. L. Clarke for his advice and unfailing help and Dr B. H. Ketchum for the early P analyses. We are also indebted to Dr R. J. Conover and Dr J. Steele for fruitful discussion of the results.

SUMMARY

It is shown that equilibrium of ^{32}P throughout the body of *Calanus* is reached only after a feeding period of about a week. The time required to liberate by excretion the amount of P present in a *Calanus* is about 20 days. Fed *Calanus* excrete at a higher rate than starved. The implications of excretion in feeding experiments using ^{32}P is discussed.

There are large differences in the total P content of individual *Calanus*. Figures are given for ripe and unripe females, fat and lean Stage V, and eggs. A high percentage, up to 55 % of the total P, may be lost by excretion during starvation.

A high proportion of the P in the food assimilated (up to 70 %) may appear within a week as eggs laid.

Liberation of P from dead *Calanus* is very rapid and is almost complete in three days.

The relation of excretion and autolysis to the P cycle in the sea is discussed.

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ADDENDUM

The Turnover of Phosphorus by *Calanus finmarchicus*

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It has been shown above that the rate of loss of ^{32}P from *Calanus* is in part dependent on the length of time of feeding on the radioactive culture prior to the start of the excretion measurements. It was also observed that the ^{32}P was excreted from *Calanus* more rapidly in fed than in starved animals after short-term feeding with labelled cultures (see Fig. 2 and Tables 1 and 2, pp. 466, 467 and 470). Finally, it was shown that the rate of loss of ^{32}P in starved *Calanus* was frequently more rapid than the loss in total phosphorus (see Table 7, p. 476). Thus the radioactive phosphorus must be presumed to pass through several different physiological or chemical 'pools' each with a different turnover time before equilibrium is approached.

In the series of experiments described, the culture medium was changed each time the ^{32}P loss from the animals was measured and, therefore, the design conforms more closely to an open than a closed system, i.e. to one in which there is a continuous flow into and out of the system with no reversible reactions in contrast to one in which there is maximum recycling. Changes in total phosphorus with feeding are sometimes positive and sometimes negative (Table 7); in the absence of any pronounced trend toward an increase or decrease in total phosphorus in the experiments, a steady state will be presumed.

In the simplest type of open system, assuming excretion from a single phosphorus pool under steady-state conditions and with no recycling, the amount of isotope in the pool would decrease according to the expression

$$X = ae^{-kt}, \tag{1}$$

where X is the amount of radioactivity at any time t, a is the initial concentration of ^{32}P and k is the turnover rate. Under these experimental conditions a semilog plot of the amount of ^{32}P in the animal against time should give a straight line with slope k. In Fig. 5, the data for fed animals from Table 2 (Expt. 9–22.xii.59), taken as percentage ^{32}P remaining in the copepod body, have been plotted on semilog paper against time. After 2–3 days the loss would seem to be linear, but over the first 24 h at least it is much more rapid than would be anticipated if only a single rate function applied. These animals were fed only 3 h on labelled phytoplankton before the excretion measurements were begun. With a longer initial labelling period, the percentage loss of ^{32}P from the bodies of the experimental animals during the first few hours after

excretion measurements were started was not so great (see Fig. 1 and Table 1). It must, therefore, be presumed that there is at least one additional pool of small size, but with a high turnover rate, that is intermediate between the external phosphorus supply and the larger, stable pool of body phosphorus. Because this pool is proportionately so small compared with the stable pool, its turnover rate can be studied with precision only if the initial labelling period is kept short.

Since the data strongly suggest that more than a single rate process is involved in the uptake and loss of phosphorus by *Calanus*, it was decided to perform the analysis with a two-compartment rate function, assuming a steady state and no recycling, such that

$$X = a_1 e^{-k_1/t} + a_2 e^{-k_2 t}, \tag{2}$$

where a_1 and k_1 represent the initial concentration and daily turnover rate for the labile pool and a_2 and k_2 are the concentration and turnover rate for the stable pool. In Fig. 5, a smooth curve has been fitted by eye to the data for animals labelled 3 h and an asymptote to the straight part of the curve extended to the ordinate. The slope of this line k_2 is the turnover rate for the slow reaction and its y intercept is equivalent to the radioactivity in a_2 at the end of the labelling period, i.e. $t = 0$. When the ordinates of this line are subtracted from the portion of the curve which does not correspond with it, a second curve describing the fast reaction is obtained with slope k_1 and y intercept a_1.

The slopes (k_i) of the curves may be determined from the expression

$$k_1 = 0{\cdot}693/T_1, \tag{3}$$

where T_1 is the half time of the reaction determined from the graph by inspection. For the fast reaction the half life T_1 is 0·375 days ($k_1 = 1{\cdot}848$) and for the slow reaction $T_2 = 13$ days ($k_2 = 0{\cdot}053$). The y intercepts $a_1 = 0{\cdot}44$ and $a_2 = 0{\cdot}56$ indicate that the amount of ^{32}P in the two pools after 3 h feeding was nearly equal.

A similar analysis procedure was carried out for the other experiments using the data for fed animals contained in Table 2. These results are summarized in Table 9. As in the case of the experiment shown in Fig. 5, when the labelling period was kept short the ^{32}P in the labile pool constituted an appreciable percentage of the total and it was possible to evaluate the rate constants (k's) for both the slow and fast reactions. However, in experiments with more than one day's labelling, over 90 % of the tracer appeared in the stable pool and only the slow reaction could be studied. Variability in the estimate for k_1 (31·8 %) was somewhat greater than that for k_2 (24·5 %) which may in part be due to the small number of measurements made during the critical first few hours after the excretion measurement was begun; however, particularly in Expts. 15–28.xii.59 and 4–16.xi.59 (Table 1), the graphic

analysis suggested that a third metabolic pool might exist intermediate between a_1 and a_2.

The values for a_1 and a_2 in Table 9 give some idea of the partitioning of the isotopic phosphorus between the two pools with different periods of feeding on labelled phytoplankton culture. However, in order to determine the relative dimensions of the two pools, it is necessary to estimate how much ^{32}P each could acquire at infinite time. At initial time the ^{32}P will all be in the

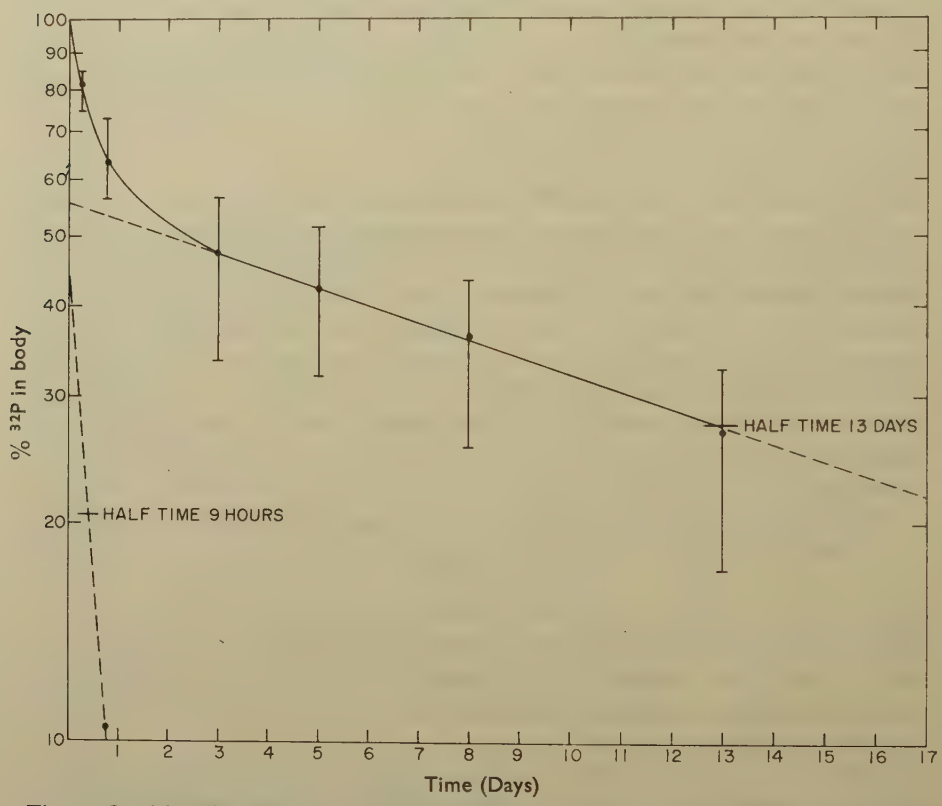

Fig. 5. Semi-logarithmic plot of the percentage of the initial burden of ^{32}P in the body of *Calanus finmarchicus* against time (data taken from Table 2, experiment of 9–22. xii. 59). The smooth curve has been fitted by eye to the mean percentage ^{32}P in the animals. Vertical lines indicate the range of individual variation. The dashed lines represent graphic extrapolations carried out using the procedure outlined in the text.

algae and will have a certain concentration in relation to the total phosphorus which determines the upper limit of radioactivity which can be acquired by each of the pools. The uptake of ^{32}P then, in each pool, behaves according to the expression

$$X_1 = a_{1\infty}\,(1 - e^{-k_1 t}), \tag{4}$$

where $a_{1\infty}$ represents the radioactive concentration at infinite time in the

ith pool and k_i is the rate constant governing uptake by this pool (for derivation of this and other formulae used, see Robertson, 1957). If the system is in a steady state with no recycling, it can be shown that the rate constants governing the uptake must equal those governing the loss from each pool. The total ^{32}P burden after 3 h or after one day's feeding on labelled phytoplankton is known for the four experiments shown in Table 10. It is also known how this radioactivity is distributed between the two pools a_1 and a_2 (see Table 9). Therefore, if the radioactivity in counts/minute in a_1 or a_2 is set

TABLE 9. VALUES FOR TURNOVER RATE (k_i) AND PROPORTION OF RADIOACTIVITY IN THE PHOSPHORUS POOLS (a_i) IN *CALANUS FINMARCHICUS*

Date of experiment	Length of labelling period (feeding with labelled phytoplankton)	Fast reaction		Slow reaction	
		Turnover rate k_1	Proportion in a_1	Turnover rate k_2	Proportion in a_2
9–22. xii. 59	3 h	1.848	0·44	0·053	0·56
15–28. xii. 59	3 h	2·081	0·375	0·037	0·625
4–16. xi. 59	1 day	0·924	0·50	0·036	0·50
24. xi.–18. xii. 59	1 day	2·081	0·38	0·069	0·62
17. xi.–1. xii. 59	7 days	—	—	0·072	0·915
1–15. xii. 59	7 days	—	—	0·048	0·965
10–26. v. 58	14 days	—	—	0·048	0·92
28. xii.–12. 59. i. 60	18 days	—	—	0·060	0·915
		Mean = 1·734 σ = 0·551 C.V. = 31·8 %		Mean = 0·053 σ = 0·013 C.V. = 24·5 %	

TABLE 10. POOL SIZE AT INFINITE TIME AND TOTAL PHOSPHORUS EXCRETION FOR *CALANUS FINMARCHICUS* LABELLED FOR PERIODS OF ONE DAY OR LESS

Date of experiment	Length of labelling period	Mean initial radioactivity (ct/min)	Size of pool a_1 (% total P)	Size of pool a_2 (% total P)	Total daily turnover of E_T (%/day)
9–22. xii. 59	3 h	32,116	2·3	97·7	9·4
15–28. xii. 59	3 h	17,926	1·3	98·7	6·4
4–16. xi. 59	1 day	21,358	6·1	93·9	9·0
24. xi.–8. xii. 59	1 day	58,556	4·5	95·5	16·0

equal to X_1 and if the corresponding values for k and for t from Table 9 are inserted, equation (4) may be solved for $a_{1\infty}$. Since the proportion of radioactive phosphorus to total phosphorus should be the same for each pool at infinite time, the relative dimensions of the two pools can now be computed as percentage of total body phosphorus. Thus in Table 10 it is shown that somewhere between 94 % and 99 % of the total phosphorus content of *Calanus* is in relatively stable form.

It would also be of considerable importance for the understanding of nutrient cycles in the sea to determine the phosphorus turnover rate for the whole animal. Since the amount of phosphorus excreted by the animal will

depend on the fraction lost per unit time from each pool and the relative amount of phosphorus in each pool, it is necessary to use the differential form of expression 2 to obtain an estimate of turnover for *Calanus*. Thus, the excretion rate

$$-\frac{d(a_1+a_2)}{dt} = E_T = k_1 a_1 + k_2 a_2. \qquad (5)$$

Values for phosphorus excretion for the four experiments where the labelling period was short are shown in the last column of Table 10. The mean turnover rate would seem to be about 10 %/day. As many members of the zooplankton community are smaller than *Calanus* and may be expected to be more active metabolically, the possibility that excretion by plankton animals contributes significantly to aquatic nutrient budgets should not be overlooked.

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THE FUNCTIONAL MORPHOLOGY OF THE BRITISH SPECIES OF VENERACEA (EULAMELLIBRANCHIA)

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(Text-figs. 1–15)

The Superfamily Veneracea comprises the three families: Veneridae, Petricolidae and Glaucomyidae (Owen, 1959). Of these the Veneridae has sixteen British species (including *Mysia undata*) representing seven genera, and the Petricolidae one, namely *Petricola pholadiformis*, an introduced North American form which has become common in some areas of the British coast (Burton, 1958). There are no British representatives of the Glaucomyidae. Members of the Veneracea inhabit every type of soft substratum and occur in all seas. Theile (1934) lists thirty-three genera. The Veneracea is therefore an important and successful group of eulamellibranchs. This paper is based on a study of twelve of the British species including representatives of all British genera except *Callista* and *Irus*. The nomenclature adopted throughout is that of Winckworth (1932, 1951).

The localities from which the various species were obtained are listed below together with brief notes on their normal habitats.

Dosinia exoleta (L.). Occasional specimens were obtained from localities near Millport. It is recorded from depths from L.W.S.T. to about 50 m (Haas, 1929–40), but in the Clyde sea area it usually occurs between 10 and 40 m, on gravelly or stony bottoms with some mud.

Dosinia lupinus lincta (Montagu) was obtained from Kames Bay, Millport, where it occurs in sand and muddy sand at a depth of 5–20 m. Larger specimens were collected from grounds offshore of the Lion Rock, Isle of Cumbrae, from fine shell gravel at a depth of 10–20 m. Haas (1929–40) says that this species is found at depths from 25 to 150 m. It is generally associated with a finer grade of substratum than *D. exoleta*.

Gafrarium minimum (Montagu) was obtained from stony or coarse gravel grounds together with *Venus casina* (L.), *V. ovata* (Pennant) and *V. fasciata* (da Costa). *V. striatula* (da Costa) was obtained from Kames Bay and from Hunterston sands, Ayrshire (Ansell, 1961 *a*).

Venerupis aurea (Gmelin) was collected from various localities on the shores of West Loch Tarbert, Argyll and Loch Sween, Argyll, where it occurs together with *V. decussata* (Gmelin). Both species were found in a narrow

belt from about L.W.N.T. to just below L.W.S.T. *V. decussata* is normally found burrowing in coarse gravel, while in the situations described here, *V. aurea* was only rarely found buried.

Venerupis pullastra (Montagu) was collected from the Cross Houses Beach, Millport, where its occurrence has been described by Quayle (1952).

Venerupis rhomboides (Pennant) was collected in small numbers off-shore of the Lion Rock, Isle of Cumbrae, at a depth of 20–30 m. This is the only species of *Venerupis* occurring in this country which is normally found at depths greater than 10 m (Haas, 1929–40).

Mysia undata (Pennant) was found burrowing in muddy shell gravel at 10–20 m depth off the Isle of Cumbrae. Specimens of *Petricola pholadiformis* (Lamarck) were kindly sent from Burnham-on-Crouch, Essex, by Mr G. D. Waugh to whom the author expresses his thanks.

Where possible, drawings have been prepared from relaxed and preserved specimens. Magnesium sulphate or propylene phenoxytol (Owen, 1955 *a*) was used as the relaxing agent. Accurate drawings were prepared first, and details of the ciliary currents added later.

Ciliary currents were studied by the use of carborundum powder grade 3 F, carmine suspensions or suspensions of the alga *Phaeodactylum*.

Where necessary sections have been prepared from tissues fixed in Bouin's fluid in sea water and embedded in ester wax (Steedman, 1947). These were stained with Ehrlich's haematoxylin and eosin, Azan, or Mallory's Triple stain.

VENERIDAE

Shell

The shell and hinge teeth of the Veneridae have been described by several authors including Forbes & Hanley (1853) and Jeffreys (1863). In general in the British species there are three well developed cardinal teeth in each valve, one or more of which are bifid. A low ridge on the anterior side is usually regarded as a reduced lateral tooth, but well developed lateral teeth are present only in the genus *Gafrarium*. The descriptions of the hinge teeth of the Veneridae given by Jeffreys (1863) are confusing; those of the left valve are described as the right and vice versa.

Owen (1953 *a*) has described the form of the shell in bivalves and he suggests that growth may be considered as the resultant of three components: (*a*) a radial component radiating from the umbones and acting in the plane of the generating curve; (*b*) a transverse component acting at right angles to the plane of the generating curve; and (*c*) a tangential component acting tangentially to and in the plane of the generating curve. The resultant shape of the shell is determined by the relative magnitude of these three components. The form of the shell valves should therefore be considered with reference to the outline of the generating curve and the form of the demarcation line (Yonge, 1955,

= normal axis, Owen, 1953*a*). Where the three components are all present they combine to produce a shell in which the demarcation line is in the form of a spiral. This is well illustrated by *Glossus humanus* (see Owen, 1953*b*), but occurs in many groups of bivalves including the Veneridae, where the direction of the tangential component is such that the umbones are inclined anteriorly. The effect of this on the ligament will be dealt with later.

The shape of the generating curve depends largely on the magnitude of the radial component around the mantle margin. Asymmetry of the generating curve about the demarcation line is increased if the region of greatest marginal increase lies anterior to, or posterior to, the demarcation line. In the Veneridae this effect is greatest in some species of *Venerupis* where the region of greatest marginal increase is situated posteriorly.

The external surface of the shell is covered with a thin clear periostracum. The structure of the various shell layers has been described by Bøggild (1930).

Ligament and mantle isthmus

The structure of the ligament in the Veneridae is characteristic and shows little variation from species to species. This account is of the ligament of *Venus striatula*, but applies equally well to the other species examined. The nomenclature used is that of Owen, Trueman & Yonge (1953), and of Yonge (1957).

The relationship of the ligament, mantle and shell valves is shown in Fig. 1, B–H. These drawings form a series taken from the extreme posterior end of the animal to a region anterior to the umbones as shown in Fig. 1, A.

Fig. 1, B represents a section taken at the extreme posterior end of the animal in the region of the siphons. Here the inner folds of the mantle edge together with part of the middle folds are fused to form the siphons, while the outer folds (*of*) and the remainder of the middle folds (m_2) on either side of the periostracal grooves remain separate. Anteriorly the middle folds fuse to form a single median ridge (*fmf*, Fig. 1, C) between the periostracal grooves (*pg*). This represents the extent of fusion of the mantle edges occurring along the whole of the dorsal side posterior to the functional ligament; there is no region of periostracal fusion.

Posterior to the ligament (Fig. 1, D) the outer surfaces of the outer mantle folds fuse (*fof*) and a thin layer of conchyolin-like material is secreted from the fused surfaces. This is the fusion layer (*fl*) which forms a secondary extension of the primary ligament posteriorly (Yonge, 1957). As growth proceeds, the fusion layer becomes split in the mid-line over the anterior region of the ligament. The outer surface of the outer mantle fold immediately posterior to the mantle isthmus secretes material on the inner surface of the fusion layer, forming the outer layer of the primary ligament (Fig. 1, E; *ol*). Finally the inner layer (*il*) is secreted by the epithelium of the mantle isthmus (Fig. 1, F; *mi*).

Owen (1953*b*) has discussed the effect of the tangential component of shell growth on the form of the ligament of *Glossus humanus*, and the same considerations apply here. In bivalves in which there is no tangential component, as in *Glycymeris*, growth of the mantle/shell anterior to the umbo takes place anteriorly. In the Veneridae, as in *Glossus*, however, growth of the valves is affected by the tangential component, which, in the region anterior to the umbo, acts in the opposite direction to the radial component. Since the tangential component is stronger than the radial component in this region

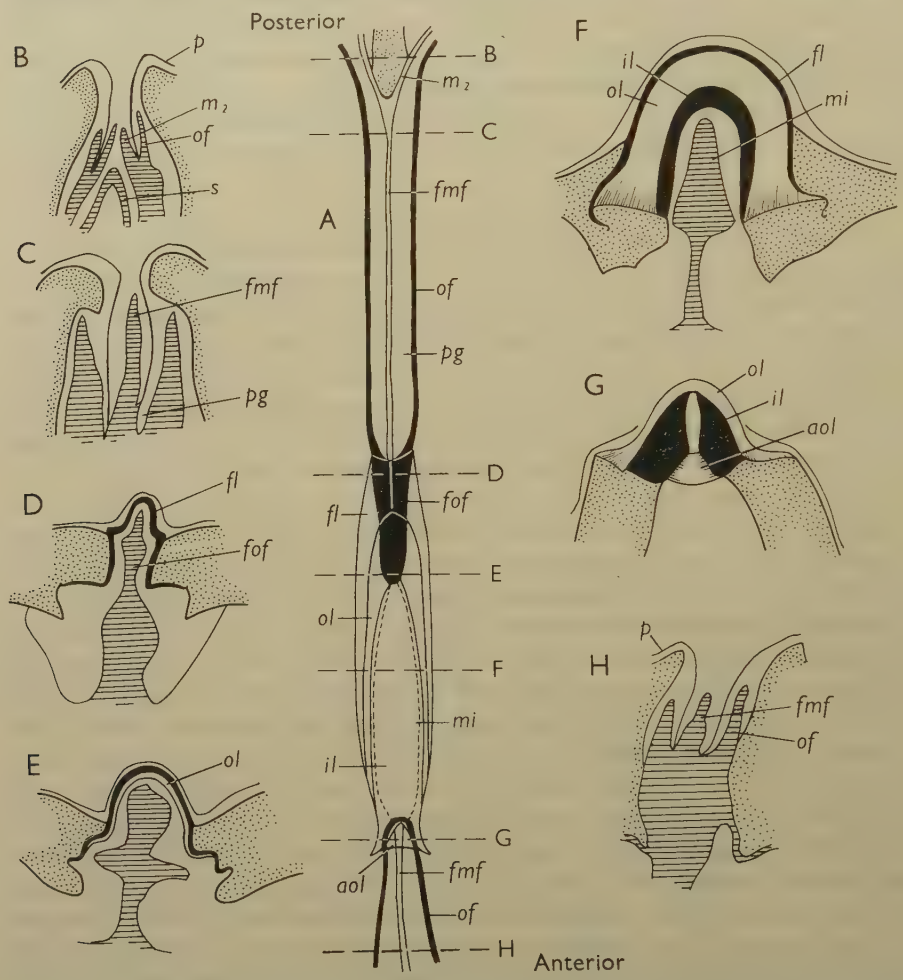


Fig. 1. *Venus striatula*: diagrams to show the relationships of the ligament, mantle and shell valves. A, Dorsal view of the ligament and mantle margin in a de-calcified specimen. B–H, Transverse sections of the ligament, shell valves and mantle. The positions of these sections are indicated in A. For lettering see p. 517.

growth of the mantle/shell takes place posteriorly, with the result that the anterior end of the ligament between the umbones is progressively split as growth proceeds (Fig. 1, A). Anterior to the umbones there is no fusion of the outer mantle folds and hence no fusion layer. The mantle edge at the anterior end of the ligament secretes an anterior outer layer between the split portions of the ligament (Fig. 1, G; *aol*).

Apart from these effects the ligament of *Venus striatula* and of other members of the Veneridae is that of a typical bivalve possessing an external, opisthodetic ligament and is basically similar to that of *Glossus humanus* (Owen, 1953*b*). In the Veneridae, as in this genus, the fusion layer is split in the mid-line over the anterior region of the ligament, the ligament is split anteriorly, and there is no region of periostracal fusion.

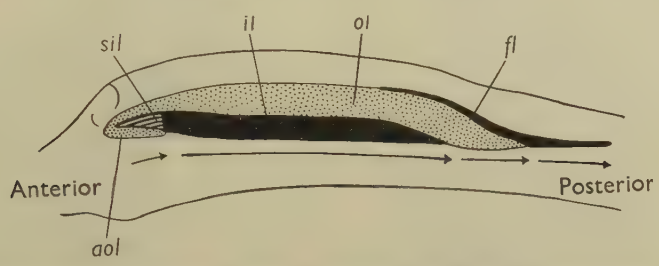


Fig. 2. *Venus striatula*: diagrammatic vertical longitudinal section of the ligament. The arrows indicate the direction of growth of each of the layers. For lettering see p. 517.

Mantle edge and mantle fusion

In the Bivalvia the mantle margins are divided into three folds (Yonge, 1957). The outer fold secretes the two outer layers of the shell. The middle fold is sensory and may carry tentacles and even eyes, while the inner fold is typically muscular and controls water movements into and out of the mantle cavity.

The form of the mantle edge in the Veneridae presents certain distinctive features. The free mantle margin is split into *four* folds: a typical outer fold which secretes the outer calcareous layer of the shell and the periostracum from the outer and inner surfaces respectively, and three others, the innermost of which is a small flap-like fold directed dorsally (Fig. 3).

The homologies of these folds with those of other bivalves might be interpreted in two ways.

Yonge (1957) has suggested that the middle fold of the mantle edge of the Veneridae is duplicated. In this case the inner fold would be here represented by the small flap (*if*). The two middle folds described here would represent the separated inner and outer surfaces of the typical bivalve mantle edge middle fold, the innermost having taken over the function of the inner fold in sealing the mantle cavity.

Alternatively, the three outermost folds may be regarded as homologous with the three folds of the typical bivalve mantle edge, and the small, flap-like, innermost fold as a fold of the inner surface of the mantle similar to those which occur in certain members of the Tellinacea (Yonge, 1949). In the Veneridae, however, they do not enclose a waste canal and have no function in the disposal of pseudo-faeces.

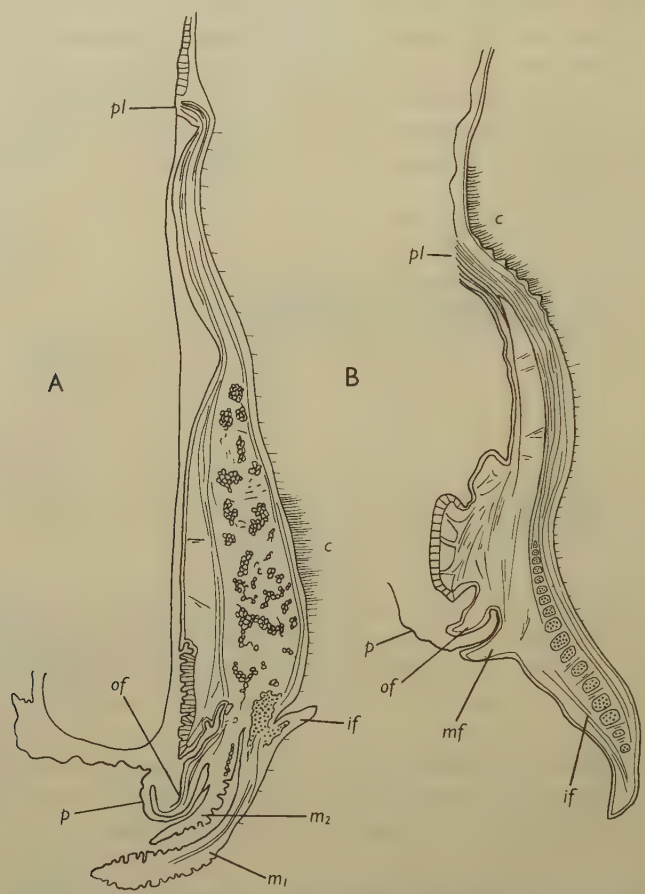


Fig. 3. Transverse sections of the ventral mantle margins of: A, *Venus striatula*; B, *Cardium norvegicum*. For lettering see p. 517.

Details of the fusion of the mantle folds anterior to the pedal gape (Fig. 4) suggest that the first of these alternatives is correct. The small, flap-like folds may be traced around the pedal gape until the folds from each mantle lobe fuse dorsal to the anterior adductor muscle. They clearly differ therefore from the folds of the inner mantle surface of some bivalves, which do not extend so far anteriorly and never fuse in the mid-line. The fusion of the middle two

folds supports the view that they together represent the middle fold of the mantle edge of a typical bivalve, since these two folds (m_1, m_2) on each side fuse together to form a single fold on each side before fusion in the mid-line occurs. It appears that the folds must be interpreted therefore as: (1) a typical outer fold, (2) and (3) duplicated middle folds representing separated outer and inner surfaces of the typical bivalve middle fold, and (4) a reduced inner fold, the normal functions of which have been taken over by the innermost part of the middle fold.

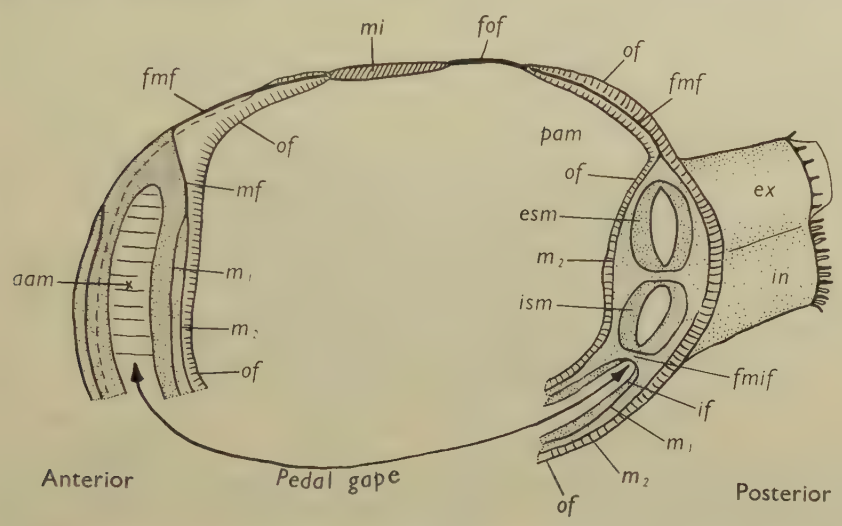


Fig. 4. Diagrammatic stereogram to show the details of fusion of the mantle folds in the Veneracea. For lettering see p. 517.

Fusion of the mantle folds posteriorly to form the siphons is of type B (Yonge, 1957). As in the Solenacea and the Cardiacea, the entire region between the ridge bordering the inner side of the periostracal groove and the siphonal tentacles is to be regarded as formed from the outer surface of the middle fold of the mantle edge; in this case from the innermost of the duplicated middle folds.

A constant feature of the siphons of the Veneridae is the presence at the proximal ends of valve-like structures—the siphonal membranes. In some species, e.g. *Venus striatula* (Fig. 5 A), these membranes are simply low ridges. In others, e.g. *V. ovata* (Fig. 5 B) and *Gafrarium minimum*, they show much greater development and appear to be capable of closing the inner apertures of both siphons. In *Venus casina* there is a double ridge at the base of the inhalant siphon. Similar siphonal membranes occur in *Pholadidea loscombiana* (see Purchon, 1955*a*), *Petricola pholadiformis* (see Purchon, 1955*b*) and *P. carditoides* (see Yonge, 1958). Kellog (1915) recorded similar structures in

many North American species, and Haas (1929–40) states that they occur at the base of the siphons in many groups of bivalves. In some species of the Tellinacea (Yonge, 1949) the inhalant siphonal membrane is associated with a covered waste canal and directs the inhalant current ventrally in the mantle cavity. In the Veneridae the opening between the membranes is normally slit-like, but in some species (e.g. *Venus ovata*) the membrane may apparently direct the current dorsally. It is probable that the membranes function in controlling the water current passing through the mantle cavity.

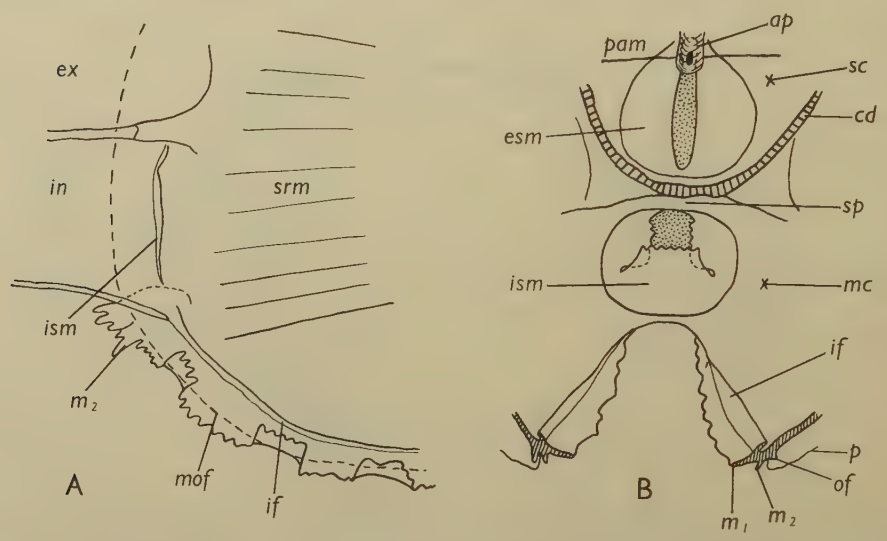


Fig. 5. Drawings to illustrate the form of the siphonal membranes in: A, *Venus striatula*; B, *Venus ovata*. A, Internal view of the base of the inhalant siphon; B, view into the base of the siphons from the mantle cavity. For lettering see p. 517.

The siphons of the species examined are all similar in structure and pigmentation. In transverse section (Fig. 6) distinct blocks of longitudinal muscle occur, separated by radial and circular muscles, and other material thought to be collagen fibres. The inner and outer surfaces of the wall are covered by a layer of cubical epithelium, with, on the external surface, a thin cuticle. No mucus-secreting cells were seen on the outer surface.

Near the ends of the siphons irregular patches and streaks of reddish brown pigment occur in the outer surface. Elsewhere the surface of the siphons is a smooth creamy white, this appearance being caused by the presence of opaque white patches on the inner surfaces.

The species vary in the length of the siphons, the extent of fusion, and the development of tentacles from the inner and middle folds of the mantle edge around the free apertures (Fig. 7). Particularly interesting in this respect is the genus *Venerupis*. In *V. pullastra* and *V. rhomboides* the siphons are fused,

apart from a small area near the tip. In *V. aurea* the siphons are fused only for half their length, whilst in *V. decussata* the siphons are almost completely separate. In all species of this genus tentacles are well developed; a ring of simple tentacles derived from the middle fold of the mantle edge surrounding both apertures, and a ring of pinnate, straining tentacles derived from the inner fold of the mantle edge around the inhalant aperture.

In all species of the Veneridae, the primary exhalant siphon, derived from the inner mantle fold (Ansell, 1961*b*), is retained at the distal end of the exhalant siphon in the adult and forms a valve which confines and directs the exhalant current.

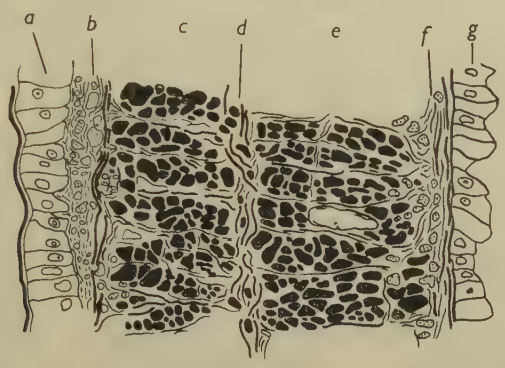


Fig. 6. *Venus striatula*: transverse section through part of the wall of the siphons. *a*, outer epithelium with thin cuticle; *b*, *f*, circular muscle, connective tissue and collagen fibres; *c*, *e*, longitudinal muscle blocks, and radial muscle strands; *d*, radial muscle strands; *g*, inner epithelium.

The differences in form of the siphons reflect important differences in the habitats of the different species. In *Venus striatula*, *Dosinia lupinus* and *D. exoleta* the tentacles surrounding the apertures are poorly developed. These three species are found typically in clean, sandy or gravelly bottoms offshore where the amount of detritus in suspension will be low. *Venus striatula* occurs in the littoral zone but only on beaches which are well sheltered and subject to little wave-action. The remaining species of *Venus* and *Gafrarium minimum* occur normally on gravelly or stony bottoms with some admixture of mud and organic debris. In these species tentacles are well developed. Finally the greatest development of tentacles and their use as a straining mechanism occurs in *Venerupis*. The members of this genus, with the exception of *V. rhomboides*, are normally littoral and occur on beaches where considerable amounts of organic debris may be brought into suspension by wave action.

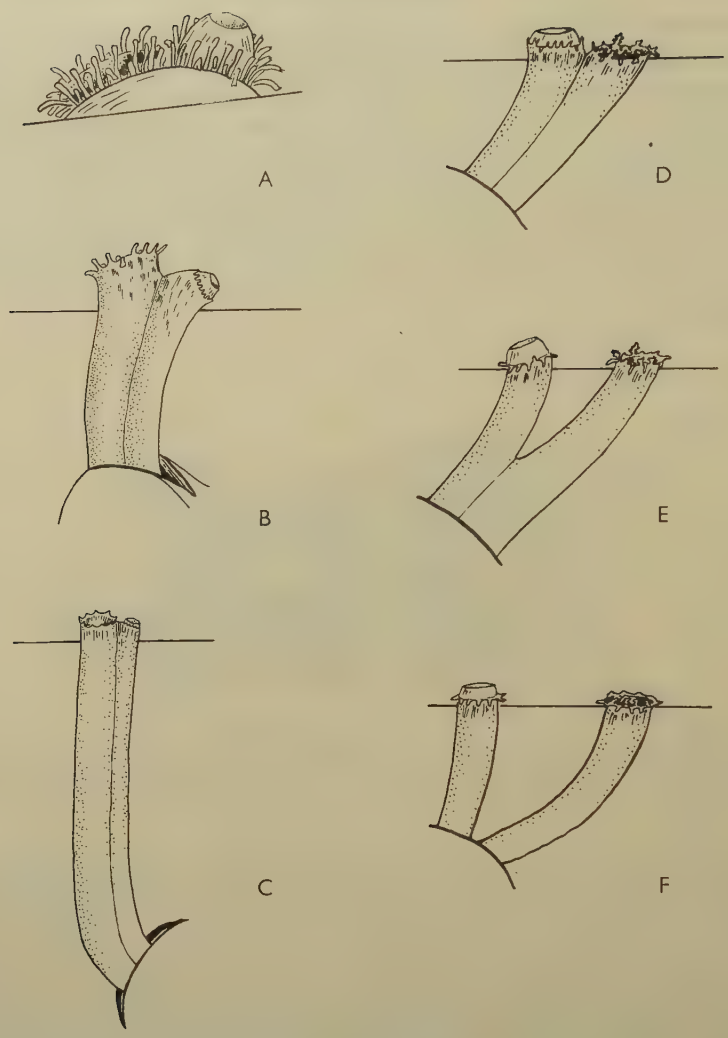


Fig. 7. The appearance of the siphons of some members of the Veneridae. A, *Gafrarium minimum*; B, *Venus striatula*; C, *Dosinia lupinus*; D, *Venerupis pullastra*; E, *V. aurea*; F, *V. decussata*.

Organs of the mantle cavity

Ctenidia

The form of the ctenidia and the general course of the currents upon them are shown in Fig. 8. Ridewood (1903) and Atkins (1937*a*, *b*) have described the form of the ctenidia and their ciliation, and present observations confirm their work.

In the Veneridae the inner demibranch is always well developed and about twice the depth of the outer. Ciliary currents on both the ascending and

descending lamellae of the inner demibranch carry particles to the free-edge where they are conveyed forward to the palps by the cilia of the marginal groove. At the extreme dorsal edge of the descending lamella particles may be carried dorsally to an oralwards current between the bases of the demibranchs.

On the outer demibranch some variation occurs between species, and even between different specimens of the same species, in the strength of the current at the free-edge carrying particles towards the palps, and in the direction in

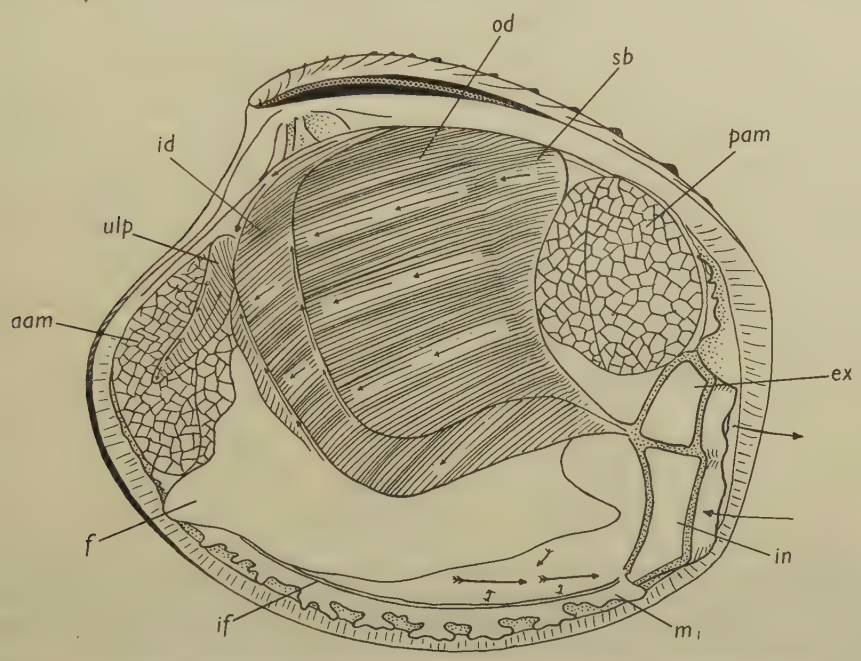


Fig. 8. *Venus casina*: disposition of the organs in the mantle cavity. For lettering see p. 517.

which particles are moved on the descending lamella (Atkins, 1937*b*). In general the currents fall into one of the two types shown in Fig. 9 which is based on two of Atkins's figures.

Groups of guard cilia are developed at the margin of the inner demibranch in all the species examined except *Venus striatula*, *Dosinia lupinus*, *D. exoleta* and *Venerupis decussata* (see Atkins, 1937*a*). Towards the posterior end of both demibranchs, long cirri are present, beating intermittently outwards from the marginal groove. Similar cirri are present in many bivalves. They possibly function in removing large particles or other obstructions from the groove.

In suspension-feeding bivalves such as the Veneridae, where normally little material enters the mantle cavity with the water current, the primary function of the ctenidia is to maintain a considerable water current and to

transfer material to the palps. Sorting by the ctenidia is of secondary importance. The large size of the ctenidia compared with the palps, and the presence of a considerable supraxial extension of the outer demibranch may be regarded as adaptations for maintaining and filtering a large flow of water. The lack of special sorting mechanisms on the ctenidia means that the bulk of material collected is transferred to the palps, the main pathway being by way of the marginal groove of the inner demibranch.

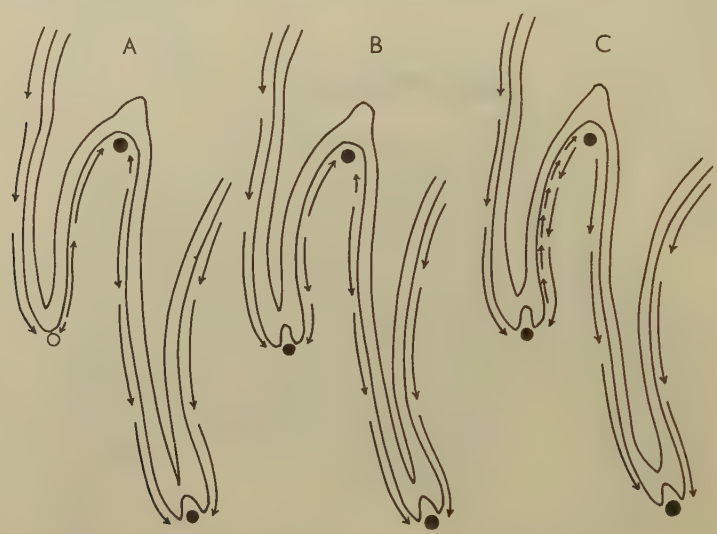


Fig. 9. Diagrams showing the form in transverse section of, and the major ciliary currents on, the ctenidia of: A, *Venus*, *Dosinia*, *Venerupis aurea*, *V. rhomboides*, *Mysia undata*; B, *Venerupis decussata*, *V. pullastra*; C, *Petricola pholadiformis*. Arrows indicate the direction of major currents, solid circles oralward currents, hollow circles incipient oralward currents.

The labial palps

The general structure of the inner ridged surface of the labial palps is shown in Fig. 8. For study of the currents, the palps were removed from freshly dissected specimens and stretched over a small piece of plasticine after being pinned out in a dissecting dish. The detailed structure of the palps, and the ciliary currents, are shown in diagrammatic form in Fig. 10.

The ciliary current on the palps may be considered under three headings (Purchon, 1955*a*):

(i) *Rejection currents*. In all species the main rejection current is on the floor and sides of the lower half of the groove. Here the cilia beat ventralwards, carrying particles out of the grooves on to unridged ventral edge where there is a strong current towards the tip of the palps.

(ii) *Acceptance currents*. Oralwards currents occur on the upper half of the folds, carrying particles rapidly forward from fold to fold.

(iii) *Re-sorting currents*. These occur on the upper halves of the ridges. Dorsalward currents occur on the upper surface of the distal shelf and ventralward currents on the crest of each ridge. These ciliary currents were similar in all the species examined.

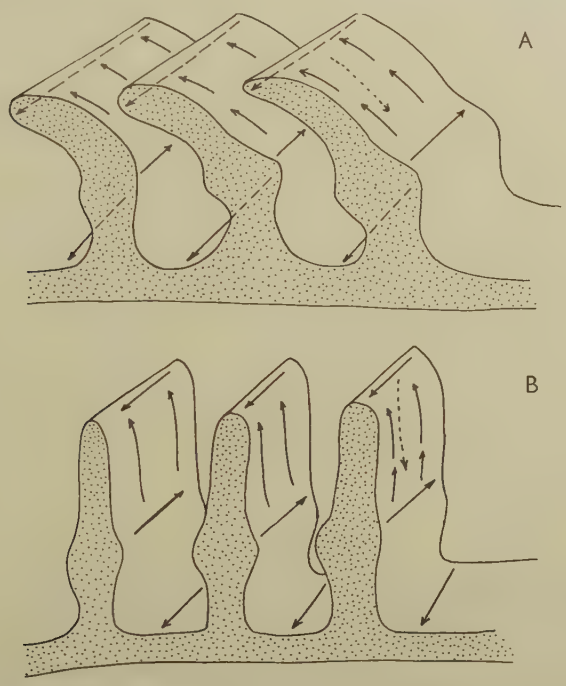


Fig. 10. *Venerupis pullastra*: diagrams to show the main ciliary currents of, and the effect of muscular activity on, the folds of the labial palps. A, Folds relaxed and overlapping, oralward currents predominate; B, folds erect, sorting and re-sorting currents predominate.

In considering the action of the labial palps in sorting food particles, muscular as well as ciliary activity must be taken into account. The palps of the Veneridae are relatively active, two main types of reaction occurring:

(i) The ridges of the inner surface may be erected or laid flat (Fig. 10).
(ii) The palps may be contracted into a spiral form bringing the ventral edge with the strong rejection current into contact with the ridged surface.

When small amounts of material are presented to the palps little sorting takes place. The ridges are relaxed and overlap and the majority of particles are carried rapidly towards the mouth by the exposed acceptance currents. Larger amounts of material elicit varying degrees of muscular activity resulting in greater efficiency of sorting. With moderate amounts of material the ridges undulate gently exposing the various re-sorting and rejection currents. Large particles fall into the depths of the grooves and are rejected while smaller particles are subjected to the sorting currents and only a small

proportion of them reach the proximal end of the palps. Excessive amounts of material brought to the surface cause the palps to react strongly, by contraction into the spiral form. Under these conditions most, or all, of the material is rejected. Selection appears to depend only on the size of the particles and not on their chemical nature.

Material which reaches the proximal end of the palps is carried towards the mouth by the cilia of the proximal oral groove. On the unridged outer surfaces of the palps the ciliary currents are directed to the dorsal edge and on to the ridged inner surface.

In considering how far the palps are adapted in each species to the particular habit three things are important: (i) the complexity of the ciliary currents and the amount of muscular activity, (ii) the number and size of the ridges on the inner surface, and (iii) the relative sizes of the palps and ctenidia. The first has been described. All the species are similar in this respect. The palps of *Venerupis* spp., however, differ from those of the other genera in being relatively larger and possessing numerous small transverse ridges. This difference may be related to the different habitats of these species; the simpler palps of the majority being adapted to a sublittoral life with little suspended matter entering the mantle cavity.

Visceral mass and foot

The foot in the Veneridae is characteristically large and wedge-shaped, being adapted for burrowing in relatively soft substrata. There is a correspondingly extensive pedal gape. In the adult there are no ciliary movements on the surface of the foot. Quayle (1949) has described the sequence of digging movements which occurs in *Venerupis pullastra*, and identical movements occur in other species, although differences occur in the timing of the sequence. Within the Veneridae, greater efficiency in digging appears to be the result of quicker repetition of the characteristic sequence and increase in the number of consecutive sequences, rather than of any change in the basic mechanism. The byssal gland is functional at settlement (Ansell, 1961*b*) but only in *Venerupis* does it remain functional in the adult.

The ciliary currents on the surface of the visceral mass differ from those of many other bivalves in being directed ventrally towards a broad antero-dorsally directed tract, so that material falling on to the surface of the visceral mass is carried towards the palps. This may result in more material reaching the labial palps since any material which is lost from the marginal groove of the inner demibranch may still be carried forward.

Ciliary currents on the surface of the mantle

The surface of the mantle is covered with short cilia, the general trend of particles carried by them being anterior and ventral towards the strong rejection tract situated near the ventral margin, and running posteriorly from a

point near the anterior adductor muscle to the base of the inhalant siphon. The cilia forming this tract are longer than those of the general mantle surface (Fig. 3 A, p. 494). Mucus is supplied to the mantle surface from cells situated below the inner epithelium and opening through ducts which run between the cells of the epithelium. The contents of these cells, together with their secretion which remains as a layer on the surface after fixation, stain blue with Alcian Blue (Steedman, 1950)—a mucin stain. Masses of pseudo-faeces collect at the base of the inhalant siphon and are rejected in the normal way.

Apart from these mucus-secreting cells and secretory cells associated with the formation of the shell and periostracum, the mantle also has numerous spaces filled with granular material. These extend in a band near the ventral margin from near the anterior adductor muscle to the siphons. The contents are acidophil, fail to stain with Alcian Blue, and appear to be similar to material occurring in the same position in *Glossus humanus* (see Owen, 1953*b*). In *Venus striatula* the contents are discharged through ducts opening between the two middle folds of the mantle edge.

Musculature

The adductor muscles are inserted into the shell in the normal position ventral to the hinge line. They are subequal, the posterior being slightly larger than the anterior, which is inserted deeper.

The anterior pedal retractor muscle is inserted posterior to the anterior adductor muscle. Some fibres radiate over the foot superficially, but the bulk radiate posteriorly in the foot internal to the posterior retractor muscle. Distally, within the foot, the fibres from either side cross over and mingle.

The posterior pedal retractor muscle is inserted into the shell anterior and dorsal to the posterior adductor muscle. The bundles from either side join below the pericardium and fibres from the right and left cross over. Where it enters the foot the bundles separate again and the fibres radiate anteriorly and ventrally in the foot.

These pedal muscles constitute the extrinsic musculature of the foot (Graham, 1934), and are of constant occurrence throughout the Veneridae. Protractor and elevator pedis muscles are never developed. In addition to the extrinsic musculature a varying amount of intrinsic musculature is also present in the visceral mass and foot: immediately below the epithelium of the visceral mass and proximal part of the foot, there are several circular muscle strands running antero-posteriorly and completely surrounding the visceral mass. In the distal part of the foot some fibres run transversely, and in the visceral mass numerous transverse fibres occur, arising in the epithelium and running either to the walls of the alimentary canal or across to the opposite epithelium. These latter are concentrated in bands which pass between the viscera, posterior to, and anterior to, the ascending coil of the hind-gut, and anterior to the combined style-sac and intestine.

Alimentary canal

General structure

A short oesophagus opens into the anterior ventral wall of the stomach, the structure of which is described in the next section. The combined style-sac and intestine passes backwards and downwards towards the heel of the foot. The mid-gut passes from the distal end of this wider tube, coils on the ventral side of the stomach, and then passes backwards to ascend posterior to the style-sac as the hind-gut. The hind-gut passes through the pericardium, penetrating the ventricle and posterior aortic bulb, and passes dorsal to the posterior adductor muscle to end in the anal papilla.

Structure of the stomach

In describing the structure and functioning of the stomach the nomenclature suggested by Graham (1949) and modified by Owen (1953*b*) and Purchon (1955*a*) will be used.

The anatomy of the stomach is in the main similar throughout the Veneridae, so that a description of one species is sufficient, points of difference being noted where they occur. *Venus casina* was chosen for study in the greatest detail, mainly on account of the greater size. Graham (1949) has described the stomachs of *V. fasciata* and *Venerupis pullastra*.

The stomach consists of the usual two parts: a large, globular anterior region, lying dorsally in the visceral mass, and a posterior elongated style-sac, with which is combined the intestine. The style-sac opens from the postero-ventral wall of the globular region and lies towards the posterior end of the visceral mass, running dorso-ventrally. The style-sac and anterior globular region of the stomach lie therefore at right angles to each other and the crystalline style, which projects into the anterior region, is curved. The appearance of the stomach exposed and opened dorsally is shown in Fig. 11.

The greater part of the left wall and roof of the globular part of the stomach is covered by the cuticular gastric shield (*gs*). Two wings of the gastric shield project into the openings of the dorsal hood (*dh*) and the left pouch (*olp*) respectively. Along the axis, the gastric shield is strengthened by a low ridge which bears a small tooth at the anterior end.

The oesophagus enters on the anterior ventral side above the openings of the right and left caeca, which lie on each side of the floor of the stomach at the anterior end. Around the opening of the oesophagus there is a low folded ridge, the anterior collecting area. The dorsal pouch opens from the right anterior wall of the stomach and curves around over the roof, the tip lying on the left side. The digestive diverticula form a dense mass of greenish brown tubules round the anterior part of the stomach, into which they open by a series of ducts via the left and right caeca and the left pouch. A number of isolated ducts may also open directly into the anterior region of the stomach. The details of the arrangement of these ducts vary from species to species.

The style-sac and intestine are only partially separated by the major and minor typhlosoles. The major typhlosole lies along the right wall, continues across the floor of the stomach, loops into the right and left caeca and ends just inside the stomach after emerging from the left caecum. The intestinal

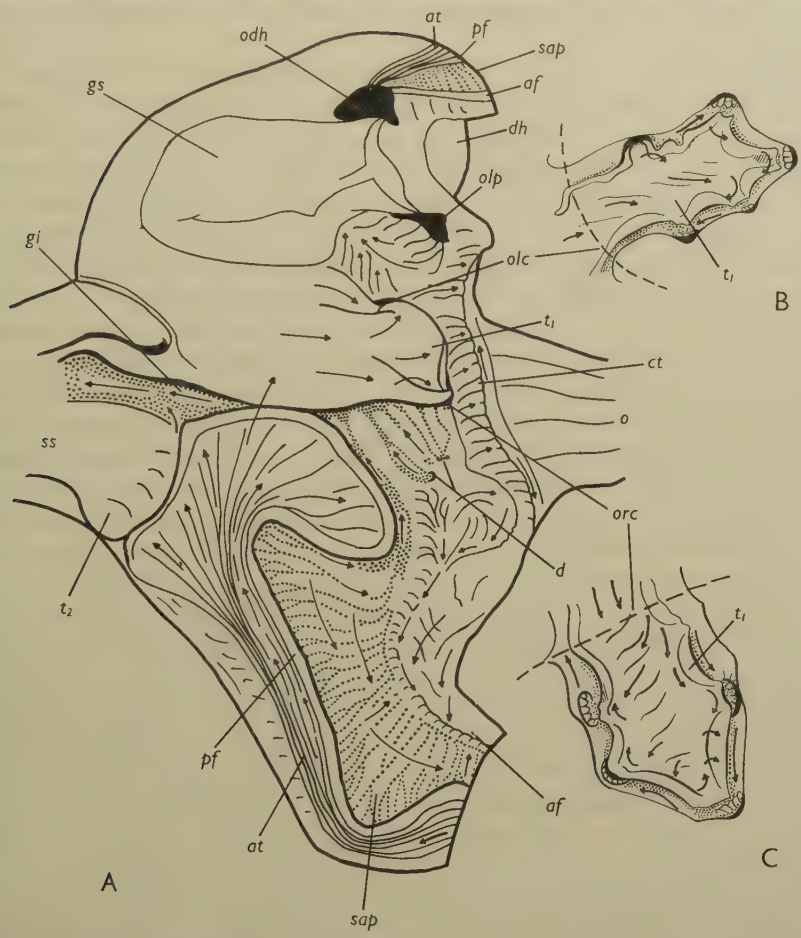


Fig. 11. *Venus casina*: drawings to show the structure and main ciliary currents of the stomach and right and left caeca. A, Stomach opened dorsally; B, left caecum; C, right caecum. Arrows indicate the direction of the main ciliary currents. For lettering see p. 517.

groove lies along the right hand side of the major typhlosole throughout this course. The minor typhlosole lies along the left wall and ends at the junction of the style-sac with the globular region of the stomach.

The right wall of the globular region of the stomach, and the walls of the dorsal hood, bear a complex system of ciliated ridges and folds, forming the posterior sorting area. This is described in the section which follows.

Sorting mechanisms of the stomach

The functioning of the bivalve stomach and digestive diverticula has been investigated and described in detail by Owen (1953*b*, 1955*b*, 1956). Observations made in the course of the present study confirm these observations made on other bivalves.

Material entering the stomach from the oesophagus is carried dorsally by the cilia of the circular groove and collects just below the opening of the left pouch (Fig. 11, *olp*). From here it is probably transferred to the posterior sorting area by the rotating crystalline style. The action of the crystalline style has not been observed in adults of the Veneridae. In the larva and young bottom stages of *Venus striatula*, however, the rotation of the style is clockwise when viewed from the anterior end of the animal (Ansell, 1961*b*) and the direction taken by particles of carmine in the style-sac of the adult confirms that the rotation is the same here. As noted by Graham (1949), the posterior sorting area is well developed in the Veneridae and lies on the right wall of the stomach extending dorsally on to the floor and sides of the dorsal hood. The movement of the cilia of the ridges and grooves of this sorting area exerts a quantitative influence on material in the stomach, fine particles being carried by cilia on the crests of the ridges into the dorsal hood (*dh*) while coarse particles fall into the grooves and are carried to the main rejection tract of the stomach, the intestinal groove. Within the dorsal hood, fine particles pass into the acceptance tract and are carried across the right wall of the stomach, over the intestinal rejection tract, and on to the floor of the stomach. In this region the intestinal groove is covered by the flap-like major typhlosole (Owen, 1955*b*). Ciliary currents on the floor of the stomach carry material to the openings of the right and left caeca (*orc*, *olc*). Particles which fail to enter the caeca are carried anteriorly by the cilia of the anterior collecting area and are returned to the general circulation by way of the circular tract (*ct*) around the oesophagus.

Within the right and left caeca the typhlosole has the form of a tube within a tube (Owen, 1955) with extensions into the openings of the ducts of the digestive diverticula. Ciliary currents on the surface of the typhlosole within the caeca serve to retain material, but are not directed into the openings of the ducts from the diverticula (Fig. 11, B, C). A similar lack of ciliary currents into the ducts of the diverticula was found in *Glossus humanus*, *Cardium edule* and *Zirphea crispata* by Owen (1955*b*) who suggests that this is a characteristic feature of the stomach of all bivalves. This view is further supported by the observations of Purchon (1955*a*) on the Pholadidae, where the currents on the surface of the typhlosole within the caeca are similarly not directed into the openings of the ducts.

Between the opening of the left pouch and that of the left caecum is a small ridged area. This extends on to the floor of the left pouch, and ciliary currents on the ridges and in the grooves direct particles dorsally to a point just outside

the opening of the left pouch and ventral to the gastric shield. No ciliary currents appear to be directed into the ducts of the digestive diverticula which open into the left pouch.

Waste material from the diverticula, together with the material rejected by the posterior sorting area, passes into the intestinal groove and hence to the intestine.

The stomach of the Veneridae, therefore, has much the same structure, and functions in the same manner, as that of a typical suspension-feeding Eulamellibranch. It is essentially similar to that of *Glossus humanus* (see Owen, 1953*b*), and of *Cardium edule* (see Owen, 1955*b*). Within the stomach food material is kept in motion by the combined action of the rotating crystalline style and the ciliated walls, and is subjected to the sorting action of the posterior sorting area. In considering the action of the bivalve stomach, some workers, and especially Purchon (1955*a*), have designated as sorting areas any region where the wall bears ridges and grooves, thus implying that such areas exert a quantitative influence on material passing over them. Many of these areas, however, direct particles indiscriminately in one direction only, and can, therefore, have no *sorting* function, but are collecting areas. In the Veneridae sorting occurs only on the posterior sorting area, where different grades of particles are carried in different directions on the ridges and in the grooves.

Pericardium

The pericardium is situated dorsally between the supraxial extensions of the outer demibranchs of the ctenidia. The ventricle (Fig. 12) is large and spherical, communicating by way of slit-like auriculo-ventricular valves with the cone-shaped, thin-walled auricles. Anterior and posterior aortae open from the ventricle and there is a large posterior aortic bulb. Blood is prevented from returning to the ventricle from the anterior aorta by a simple, annular valve at the junction of the two. The rectum runs through the ventricle and the posterior aortic bulb.

White (1942) has described the position of the pericardial glands in the Bivalvia, including several members of the Veneridae. They may lie either in the pericardial investment of the auricles (auricular pericardial glands), or in the dorsal and ventral anterior regions of the pericardium and neighbouring areas of the mantle (pericardially situated *pg*). In the Veneridae the glands occur in both these positions in some species (*Gafrarium*) while in others they appear to be restricted to the pericardial situation. In all species, however, the greatest development of the glands is in the latter position.

The kidneys of the Veneridae lie dorsal to the posterior adductor muscle and are partially overlain by the posterior aortic bulb. The two kidneys communicate by way of a large median aperture. The reno-pericardial pores open into the posterior ventral corners of the pericardium.

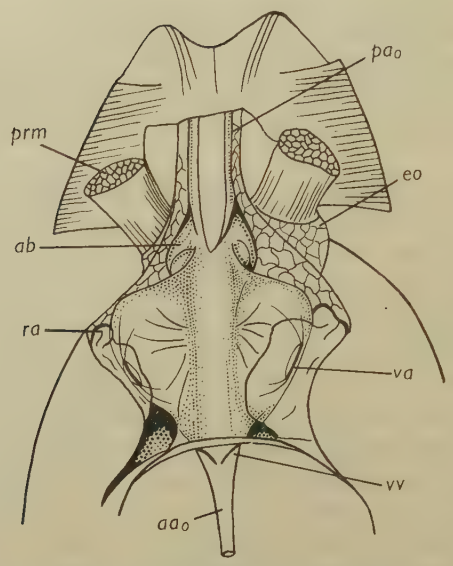


Fig. 12. *Venus striatula*: dorsal view of the contents of the pericardium. For lettering see p. 517.

Nervous system

The nervous system has been briefly studied by dissection of preserved material of *Venus striatula* only.

The cerebral ganglia are of simple, fusiform shape and lie behind the anterior adductor muscle. The cerebral commissure passes transversely close to the posterior dorsal margin of this muscle. The cerebro-pedal connective passes obliquely backwards through the anterior pedal retractor muscle and enters the anterior face of the pedal ganglion. The cerebro-visceral connective penetrates the base of the anterior pedal retractor muscle and passes back through the tissues of the visceral mass. A large nerve on each side runs round the ventral side of the anterior adductor muscle and branches to supply the anterior and ventral regions of the mantle edge. Other nerves run to the anterior adductor muscle and the dorsal region of the visceral mass.

The pedal ganglia are fused to form a large, rounded ganglion which lies in the mid-line ventral to the coils of the intestine. Two large nerves pass ventrally on each side into the foot.

The visceral ganglia lie on the anterior surface of the posterior adductor muscle. Three main nerves leave these ganglia on each side: a ctenidial nerve, a nerve to the posterior adductor muscle and a nerve to the siphonal process and posterior part of the mantle. This last-named nerve divides ventral to the posterior adductor muscle to give separate branches to various regions of the mantle (Fig. 13).

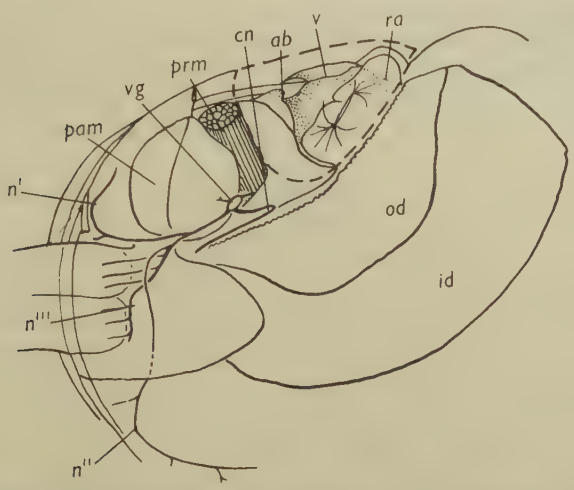


Fig. 13. *Venus striatula*: lateral view of the contents of the pericardium and the posterior part of the nervous system. For lettering see p. 517.

Reproductive system

The genital aperture lies on the posterior dorsal surface of the visceral mass, and opens into the epibranchial space above the inner demibranch. In the ripe condition, the gonad occupies most of the space between parts of the alimentary canal in the visceral mass, but does not extend into the mantle (Ansell, 1961 *a*).

MYSIA UNDATA

The systematic position of *Mysia undata* has been the subject of some disagreement in the past, being placed by some authors in the Veneridae, and by others in the Petricolidae. It is therefore considered separately here and the systematic position discussed.

The shell of *Mysia undata* is equivalve and inequilateral. The shape is suborbicular (Fig. 14). The shell is thin and fragile and the periostracum transparent. Internally the adductor muscle scars are well marked, the posterior being slightly larger than the anterior. The pallial line is distant from the edge of the shell and sinuous. There is a deep pallial sinus, extending about two-thirds of the way to the umbones. The valve margins are not crenulated.

The hinge is well developed, with three cardinal teeth in the left valve, the centre one of which is bifid. In the right valve there are two cardinal teeth, the posterior one being bifid. The ligament is identical in structure with that described for the Veneridae.

The mantle margins are free ventrally forming a large pedal gape through which the well developed muscular foot may be protruded. Posteriorly the

mantle margins fuse to form the siphons, the fusion being of type B (Yonge, 1957). The siphons are long and completely separate, the exhalant slightly shorter than the inhalant. The opening of the inhalant siphon is ringed with a double row of simple tentacles, while the exhalant has a single row of short

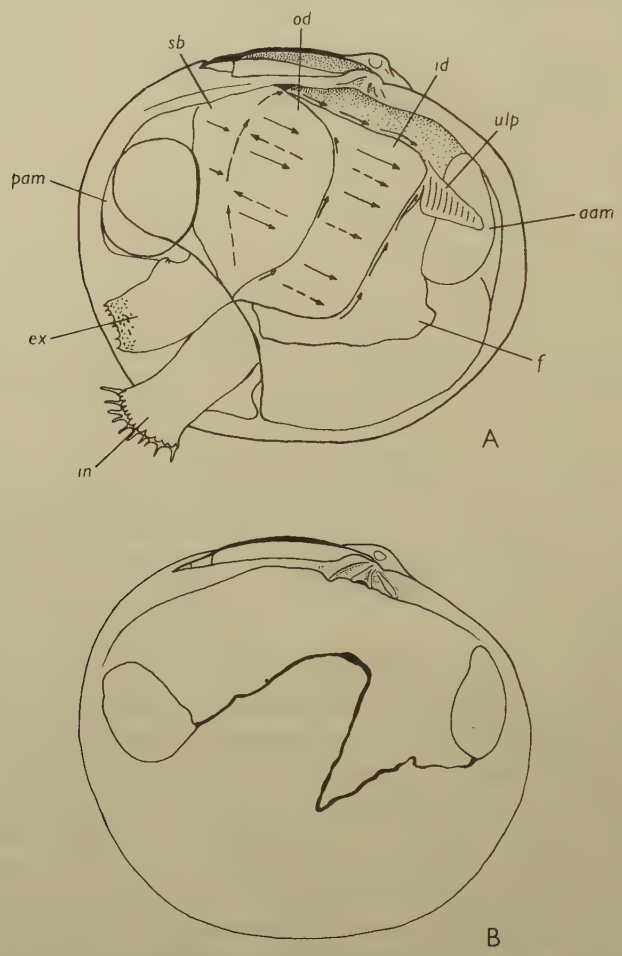


Fig. 14. *Mysia undata*: A, Disposition of the organs in the mantle cavity; B, internal view of the left shell valve. For lettering see p. 517.

tubercles. In life the siphons are protruded a little above the substratum and undergo little movement except when disturbed. *Mysia undata* is purely a suspension-feeder—a view which is confirmed by the structure of the stomach, which does not differ in essentials from that of the Veneridae.

The organs of the mantle cavity are similar to those of *Venus*. The outer demibranchs of the ctenidia are about half the length of the inner. There is a

large supra-axial extension. Ciliary currents on the ctenidia are of type C I (*b*) (Atkins, 1937*b*), with well-marked oralward currents at the free edge of the inner demibranch and at the axis of the two demibranchs, and a weak oralwards current at the free edge of the outer demibranch. Guard cilia are present. Ciliary currents on the inner surface of the mantle, the visceral mass, and the palps, are similar to those of *Venus*.

Internally there are no important differences in structure between *Mysia* and undoubted members of the Veneridae.

The main feature which *Mysia* has in common with the Petricolidae is the form of the siphons which are, in both, elongated and separate. In other features, notably in the type of ctenidial currents and in the form of the hinge teeth, *Mysia* resembles members of the Veneridae. In view of the close relationship between the Petricolidae and the genus *Venerupis* of the Veneridae, discussed later, it is suggested that *Mysia* should be classified in the Veneridae rather than the Petricolidae. The number of hinge teeth is not regarded as important in this connexion since, although all the British species of the Veneridae have three teeth in each valve, foreign genera occur in which the number is different, e.g. *Gemma* and *Cyclina* with three teeth in the left valve and two in the right (Adams & Adams, 1858).

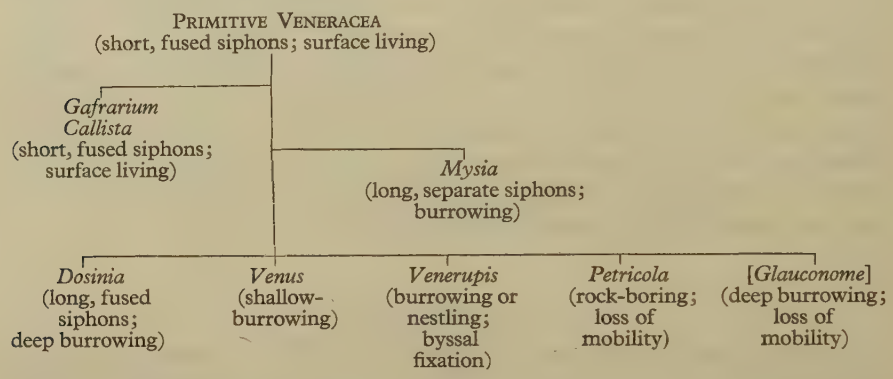
DISCUSSION

From this account of the functional morphology of the Veneracea it appears that within this Superfamily the Veneridae form a central family of closely related forms, mostly living close to the surface in relatively soft substrata, and all with a suspension-feeding habit, but without many special adaptations. This lack of special adaptations may itself be connected with the habit and habitat of the animals since under suspension-feeding conditions much of the material entering the mantle cavity is normally utilizable as food, making adaptations of the ctenidia and mantle, such as are found in deposit-feeders, unnecessary. Further, Owen (1959) has suggested that many of the adaptations of more specialized suspension-feeders (Solenidae, Myidae) are connected with a deep-burrowing habit and loss of horizontal mobility, whereas the Veneridae retain their mobility and are in general shallow-burrowing forms. Among the suspension-feeding Bivalvia, the Veneridae may be regarded as a group which has evolved along unspecialized lines. Their world-wide distribution on all types of substrata indicates their great success.

In considering the relationships of the Veneracea the most important anatomical features are those connected with the burrowing and suspension-feeding habits, notably the form of the siphons and foot, and the structure of the stomach. Certain members of the Veneridae, such as *Gafrarium* and some species of *Venus*, have short, fused siphons. Such siphons may be regarded as primitive in the Veneracea so that within the Eulamellibranchia

related forms must be sought among those groups with similar siphons. Such groups include the Astartacea, Isocardiacea and Cyprinacea. Of these the Isocardiacea and Cyprinacea show many points of similarity with the Veneracea, especially with the genus *Gafrarium*.

Within the Veneracea the least specialized condition is found in the genera *Gafrarium* and *Callista*, which have short fused siphons associated with a surface-living habit. Only slightly more specialized is *Venus*, the members of which range from near surface-living forms, like *V. fasciata*, to deeper-burrowing forms like *V. striatula* with longer siphons. Among the other members evolution appears to have followed a number of lines, one possible course being indicated below:



The genus *Dosinia* has diverged from the main group and is adapted for what is a relatively deep-burrowing habit when compared with a typical venerid such as *Venus casina*. This end-point has been reached by a different route from that taken by other deep burrowers. In these, burrowing is associated with elongation of the shell by a shift in the location of the zone or zones of greatest marginal increase away from the region where the demarcation line meets the shell edge. The animal lies vertically in the substratum with the posterior end uppermost and the foot is protruded anteriorly (*Ensis*) or is reduced (*Mya*). In contrast to these the shell of *Dosinia* is almost circular, and normally lies with the ligament more or less parallel to the surface. The foot is protruded ventrally in digging, and the anterior and posterior sets of pedal muscles perform equal work in the digging process. Burrowing is possibly assisted by the flattened lunule acting as a pressure plate preventing the animal from moving upwards as the foot is extended. The normal position of *Dosinia* in life is shown in Fig. 15 together with that of *Mya* and *Ensis* for comparison.

The two families, the Glaucomyidae and the Petricolidae, show the results of divergence from the central venerid type in two different directions, which have recently been discussed by Owen (1959) and Yonge (1958) respectively.

Glauconome is adapted by the possession of long, united siphons, extensive fusion of the mantle margin ventrally and reduction of the foot, to a deep-burrowing life in soft substrata. It presents a further contrast with *Dosinia*. The Petricolidae, as represented by *P. pholadiformis* (see Purchon, 1955) and *P. carditoides* (see Yonge, 1958), are adapted to a rock-boring habit and have long separate siphons, and ventral mantle fusion. Both groups have special adaptations of the ctenidia allowing them to deal with particles of sand and mud entering the mantle cavity, and both possess shells which are elongated by displacement of the zone of greatest marginal increase posteriorly. As Owen (1959) has suggested, members of the genus *Venerupis* show features in common with both these groups.

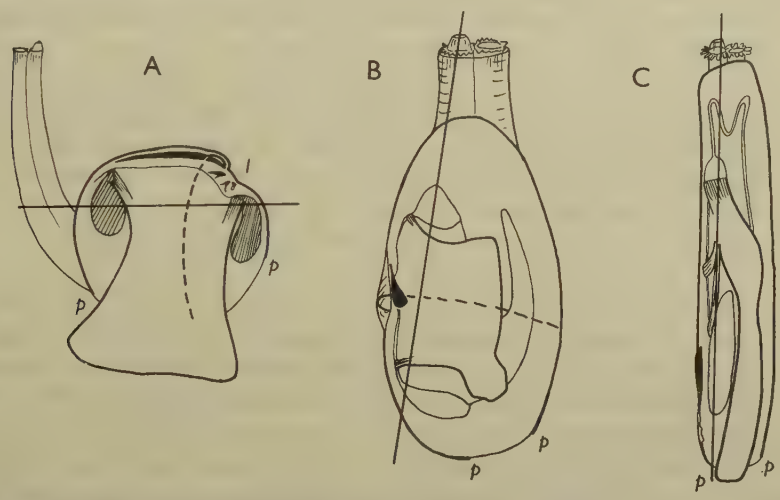


Fig. 15. Diagram to show the normal position in life of: A, *Dosinia*; B, *Mya*; C, *Ensis*. The extent of the pedal gape, the antero-posterior axis through the mouth and anus, and the demarcation line are shown.

The evolution of the rock-boring habit in bivalves has taken place along several lines. Yonge (1951) suggests that it would appear to have been preceded by specialization, in some cases for byssal attachment in crevices, i.e. 'nestling', in others for deep-burrowing into soft substrata. If the Petricolidae are regarded as evolved from a *Venerupis*-like ancestry, as seems likely, the possibility exists of the boring habit having been acquired by either of these two routes, since within the genus *Venerupis* both relatively deep burrowing (*V. decussata*) and nestling (*V. pullastra*) forms exist.

The Petricolidae are less specialized for boring than are many other groups. The main adaptations consist of the elongation of the shell posteriorly, the development of sharp ridges on the shell especially at the anterior end, and elaboration of the ciliary currents of the ctenidia. These adaptations are developed to their fullest extent in *Petricola pholadiformis* (see Purchon, 1955*b*),

and in other members the link with the genus *Venerupis* of the Veneridae may be seen more clearly. In *Petricola lapicida* the shell is similar to that of *Venerupis*, but with serrations developed especially at the anterior end, where they form a rough, jagged border to the shell (Otter, 1937). Boring is mechanical as in all the Petricolidae, but the burrow is shallow. Otter considers that the opening and closing of the shell valves would be sufficient to enlarge the burrow as growth proceeds. The internal anatomy of *Petricola lapicida* has not been described in detail and it would be interesting to see how it compares with other members of the Petricolidae, especially in regard to the ciliary currents of the ctenidia. In the Petricolidae, as in *Hiatella* (see Hunter, 1949), the byssus is not used in burrowing by the adult, as it is in *Botula* and to a lesser extent in *Lithophaga* (see Yonge, 1955). The burrowing movements are developed from the normal locomotory activities of the animal and more especially from the contractions of the adductor muscles concerned in cleaning the mantle cavity. The well developed hinge teeth and the long ligament make rocking movements of the shell valves, such as occur in the Pholadidae (Purchon, 1955*a*), impossible. This evidence suggests that the boring habit of the Petricolidae has been made possible by the pre-adaptation of *Venerupis*-like ancestors to a nestling habit rather than to deep-burrowing.

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SUMMARY

The functional morphology of twelve British species of the Veneridae is described. The systematic position of *Mysia undata* and evolution within the Veneracea are discussed.

Structurally, the members of the family Veneridae show a lack of special adaptations. The ability to move horizontally as well as ventrally in soft substrata is retained; the pedal gape is large and the foot well developed. The siphons are relatively short and fused. The food-collecting mechanisms of the mantle cavity are largely unspecialized and function in maintaining a considerable flow of water, and retaining as much as possible of the suspended material. Members of the Veneridae show a considerable uniformity of structure, but trends of evolutionary change may be distinguished. The

genera *Gafrarium* and *Callista* are least specialized. The genus *Dosinia* has evolved along an independent line and is specialized by the possession of a stream-lined, circular shell, deep lunule, and greatly elongated siphons, for a relatively deep-burrowing habit. Within the superfamily Veneracea major lines of adaptive evolution have given rise to the rock-boring Petricolidae and the deep-burrowing Glaucomyidae, and the genera *Venerupis* and *Irus* show affinities with both these families.

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List of abbreviations used in figures

aam anterior adductor muscle
aao anterior aorta
ab posterior aortic bulb
af anterior fold
at acceptance tract
aol anterior outer layer of the ligament
ap anal papilla
c cilia of the main rejection tract of the mantle
cd ctenidia
cn ctenidial nerve
ct circular tract
d isolated ducts from the right digestive diverticulum
dh dorsal hood
eo kidney
esm exhalant siphonal membrane
ex exhalant siphon
f foot
fl fusion layer of the ligament
fmif fused middle and inner folds of the mantle
fmf fused middle folds of the mantle edge
fof fused outer folds of the mantle edge
gi intestinal groove
gs gastric shield
id inner demibranch
if inner fold mantle edge
il inner layer of the ligament
in inhalant siphon
ism inhalant siphonal membrane
l lunule
m_1 innermost middle fold of the mantle edge
m_2 outermost middle fold of the mantle edge
mc mantle cavity
mf middle fold of the mantle edge
mi mantle isthmus
n', n'', n''' nerves to the mantle and siphons
o oesophagus
od outer demibranch
odh opening of the dorsal hood
of outer fold of the mantle edge
ol outer layer of the ligament
olc opening of the left caecum
olp opening of the left pouch
orc opening of the right caecum
p periostracum
p–p extent of the pedal gape
pam posterior adductor muscle
pao posterior aorta
pg periostracal groove
pf posterior fold
pl pallial line
prm posterior pedal retractor muscle
ra right auricle
s siphons
sap posterior sorting area
sb supra-axial extension of the outer demibranch
sc suprabranchial chamber
sil split portion of the inner layer of the ligament
sp siphonal septum
srm siphonal retractor muscle
ss style sac
t_1 major typhlosole
t_2 minor typhlosole
ulp upper labial palp
v ventricle
va auriculo-ventricular valve
vg visceral ganglion
vv valve at the origin of the anterior aorta

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FURTHER OBSERVATIONS ON THE FINE STRUCTURE OF *CHRYSOCHROMULINA MINOR* AND *C. KAPPA* WITH SPECIAL REFERENCE TO THE PYRENOIDS

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(With 24 Figures on Plates I–VI)

Apart from the external appendages (flagella and haptonema), for which effective methods of study are now standard, the most difficult body in cells of the Chrysophyceae to investigate with the light microscope is the pyrenoid. The reason for this is mainly that the criteria available for recognizing pyrenoids in living cells of this group are all negative, i.e. a pyrenoid is a colourless region in a small and often rather pale plastid which does not stain with any of the usual reagents giving diagnostic colour reactions with other cell components. This contrasts markedly with the state of affairs in the Chlorophyceae for which pyrenoids, when present, are conspicuous and easily demonstrable by positive criteria such as presence of starch. Nevertheless, the existence of a specialized storage area within or attached to plastids of the Chrysophyceae has been suggested by more than one light-microscopist (for literature, see Parke, Manton & Clarke, 1958), and the electron microscope has provided the strongest possible supporting evidence for this supposition.

This notwithstanding, the difficulty in detecting pyrenoids at will with the light microscope is a real one, and until it can be circumvented by chemical knowledge leading to a diagnostic colour reaction, or in some other way, the electron microscope remains an indispensable instrument for establishing the basic facts. This has already been done to the extent of revealing that two rather different types of pyrenoid can be encountered among the array of species so far described under the comprehensive genus *Chrysochromulina*. In one species, *C. chiton*, which was also the first for which the results from thin sections were available at the time of the taxonomic description (Parke, Manton & Clarke, 1958), the pyrenoid is a subspherical body attached to the inner face of a plastid by a narrow neck and densely filled with electron opaque storage material traversed by a few apparently empty canaliculi. In another species, *C. strobilus* (Parke, Manton & Clarke, 1959), a deeply immersed storage region also traversed by canaliculi was found in the centre of each plastid, the canaliculi in this case being traceable as the inner extensions of some of the plastid lamellations proper, which in the storage region become converted into closely paired tubes. Other examples of pyrenoids of this

type have subsequently been added (notably *C. ericina*, *C. alifera* and *C. ephippium* in Manton & Leedale, 1961 *a*, and *C. brevifilum* in Manton, 1961) but no others resembling *C. chiton*. The apparently isolated condition of this species is therefore a stumbling block to any unified treatment of the genus as a whole, and this difficulty cannot be resolved without further information.

Much new information has now come from a re-investigation of two of the earliest studied marine species of *Chrysochromulina*, originally described without the aid of sections (Parke, Manton & Clarke, 1955). Both *C. minor* and *C. kappa* differ from the other species enumerated above in having a smaller average cell size and a considerably shorter haptonema. This appendage is indeed so short as to be actually less than the flagella in length in a majority of cells; it is therefore superficially more like a flagellum than in any of the better known species. In addition, bodies thought to be pyrenoids but outside the plastids had been somewhat tentatively described for each, the fullest account being that given for *C. kappa* in terms as follows (Parke *et al.*, 1955, p. 589). 'A conspicuous refringent body, perhaps equivalent to the so-called pyrenoid of some authors, is situated near the centre of the inner face of each chromatophore. These bodies vary in size and sometimes appear to be surrounded by non-refringent material. They appear to divide at the same time as the chromatophores, but it is not known whether they are actually attached to the inner face....' These various points of uncertainty are the special reasons for selecting these two species for further study, and in the presentation of the new observations reference to other matters of interest will only be made incidentally, as opportunity permits, within the framework of a primary interest in pyrenoids and haptonemata.

The technical methods employed are exactly as on previous occasions except that for *C. kappa* a shortening of the fixation time from 1 h to 10 min gave greatly improved results. The details for the use of anoptral contrast for the few supplementary observations on living cells will be found in Manton & Leedale (1961 *a*). Finally the source of material has in each case been the type culture supplied from Plymouth by Dr Parke. The reference numbers in the Plymouth collection are K for *C. kappa* and 52 for *C. minor* as explained in Parke *et al.* (1955).

We are grateful to the Development Commission for financial assistance, to Dr M. Parke for supplying cultures and reading the manuscript, and to Mr K. Oates and Miss Sheila Wright for help in preparing the plates for publication and in many other ways.

NEW OBSERVATIONS ON *CHRYSOCHROMULINA MINOR*

It will be convenient to consider *C. minor* first since, although only slightly smaller than *C. kappa* (compare, for example, Pl. I, figs. 1 and 2), the internal structure is a little simpler and more easily described. Except for these two

figures it will also be convenient to deal first with the electron microscopy of sections since understanding of the latter has been, in our experience, essential for the effective further use of the light microscope.

In comparison with the small cell size of this organism the pyrenoids when seen with the electron microscope are large and conspicuous. In transverse section (Pl. III, fig. 13; Pl. IV, figs. 16, 18) the profiles are approximately circular with an average diameter of the order of 1 μ. When cut longitudinally the pyrenoids are clearly ovate, slightly narrowed at the base and commonly curved to lie along one face of the subtending plastid (Pl. II, fig. 12). It is not easy to distinguish a median longitudinal section from an oblique or tangential one, and therefore the maximum length is slightly uncertain but is of the order of 2 μ.

The base of a pyrenoid is attached near the centre of the inner face of the subtending plastid. The most critical evidence for this position is a series of transverse sections through the point of attachment from which two sample levels are reproduced in Pl. IV, figs. 17, 18.

The compound surface-membrane of the plastid can be seen in longitudinal sections to continue without interruption over the surface of the pyrenoid. Internally a few of the lamellations from the pigmented region of the plastid continue into the storage region (see especially Pl. II, fig. 11). Here they may yield circular or apparently cylindrical profiles (Pl. II, fig. 12; Pl. IV, figs. 16, 18) according to the plane of section, or, in early division stages (e.g. Pl. II, fig. 10), more contorted shapes. Occasionally an array of closely packed lamellations may line the surface of a pyrenoid (e.g. Pl. III, fig. 13, on the side touching the nucleus). The interpretation of this feature is uncertain though it is possible that it is also in some way connected with an impending division.

The relation of the pyrenoids to other cell organelles, notably the nucleus, is of some interest. In this species the nucleus is commonly pressed closely to the surface of a plastid (Pl. III, fig. 13; Pl. IV, figs. 15, 16 and 18) conforming exactly to the underlying shape whether concave (Pl. IV, fig. 18) or convex (Pl. III, fig. 13; Pl. IV, fig. 16). If it lies near to a pyrenoid the nucleus may become indented by it to a modest extent (e.g. Pl. IV, fig. 16), though there is no suggestion that this is more than incidental.

The nuclear surface is very intimately involved in these contacts. This is perhaps most clearly seen in Pl. III, fig. 13, although at higher magnifications the sections of Pl. IV, figs. 16 and 18 show it at least as well. The perinuclear space separating nuclear contents from cytoplasmic contents (except where it is bridged by a 'pore') is continuous, but where the nucleus abuts on to the surface of either a plastid or a pyrenoid the membrane bounding the cytoplasmic side of the perinuclear space disappears. At the point of junction with the free part of the nuclear surface, continuity can be traced between the membrane bounding the cytoplasmic side of the perinuclear space and the

Explanation of Plates I–IV

Plate I

Photographs of living cells taken with anoptral contrast, × 2000

Fig. 1. *Chrysochromulina kappa*. A swimming cell with extended haptonema and beating flagella.

Fig. 2. *C. minor*. A swimming cell with extended haptonema.

Figs. 3, 4. *C. minor*. Two different focal levels through a cell to show the emergence of the two flagella (fig. 3) and a profile view of the plastids and pyrenoids (fig. 4).

Fig. 5. *C. minor*. Another cell showing the attachment of the pyrenoids (*Py*) to the inner face of each plastid.

Fig. 6. *C. minor*. Partly flattened cell showing the nucleus as a dark vesicle between a bright fat body and the two facing plastids with their pyrenoids.

Figs. 7–9. *C. minor*. Three successive stages of flattening by drying out of the preparation. Fig. 7 shows the two plastids with pyrenoids (*Py*) in face view, the position of the nucleus (*N*), the leucosin vesicle (*L*), two fat bodies (*f*) and the flagella; in fig. 8 the pyrenoids have burst, each leaving a bright granule; in fig. 9 striations have become visible within the considerably distorted plastids, the leucosin vesicle (*L*) is still unburst.

Plates II–IV. Electron micrographs of *Chrysochromulina minor*

Plate II

Fig. 10. Section showing a lobed pyrenoid preparatory to division. Micrograph B650, × 20,000.

Fig. 11. Section showing a pyrenoid attached to each of two plastids or plastid halves. Micrograph B582, × 15,000.

Fig. 12. Section containing a longitudinally cut pyrenoid (*Py*), two plastids (*P*), a mitochondrion (*m*), a small piece of nucleus (*N*) and vesicle (*x*) containing a sectioned scale shown at a higher magnification in top inset. A section of scales on the body surface in the region of the arrow shown at a higher magnification in bottom inset. Micrograph B260, × 20,000; insets × 40,000.

Plate III

Fig. 13. Section to show the relation of the nucleus (*N*) with the surfaces of a plastid (*P*) and a pyrenoid (*Py*); details of the nuclear envelope and pyrenoid surface membrane resolved; a section of this cell at another level in fig. 15. Micrograph B1361, × 40,000.

Fig. 14. Three profiles from different parts of a section of a coiled haptonema showing in each case the three concentric membranes (the outer one distended) surrounding seven tubes or fibres. Micrograph B274, × 60,000.

Plate IV

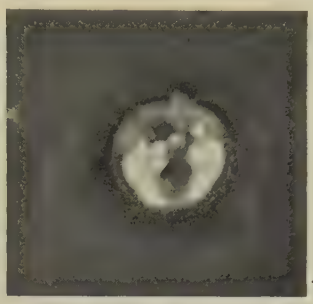
Fig. 15. Another section of the cell of fig. 13 showing the attachment of the pyrenoid to the plastid. Micrograph B1376, × *c.* 12,000.

Fig. 16. Section near the flagellar pole showing a nucleus (*N*) pressed closely to the surface of a plastid and pyrenoid. Micrograph B619, × 20,000.

Figs. 17, 18. Sections from two different levels through the same cell, fig. 17 tangential and showing the base of a pyrenoid entering a plastid centrally, fig. 18 showing the nucleus (*N*) between the pyrenoid (*Py*) and the concave inner surface of one part of the plastid which is here transected twice; two mitochondrial profiles (*m*) also shown. Micrographs B1354, × 10,000 (fig. 17), B1351, × 25,000 (fig. 18).

1

3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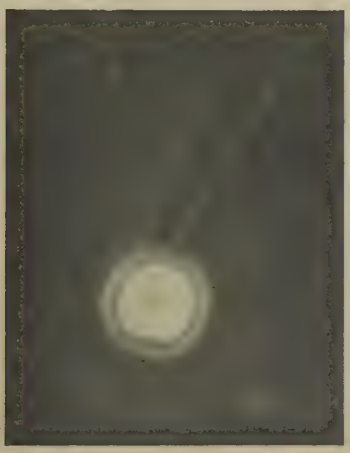
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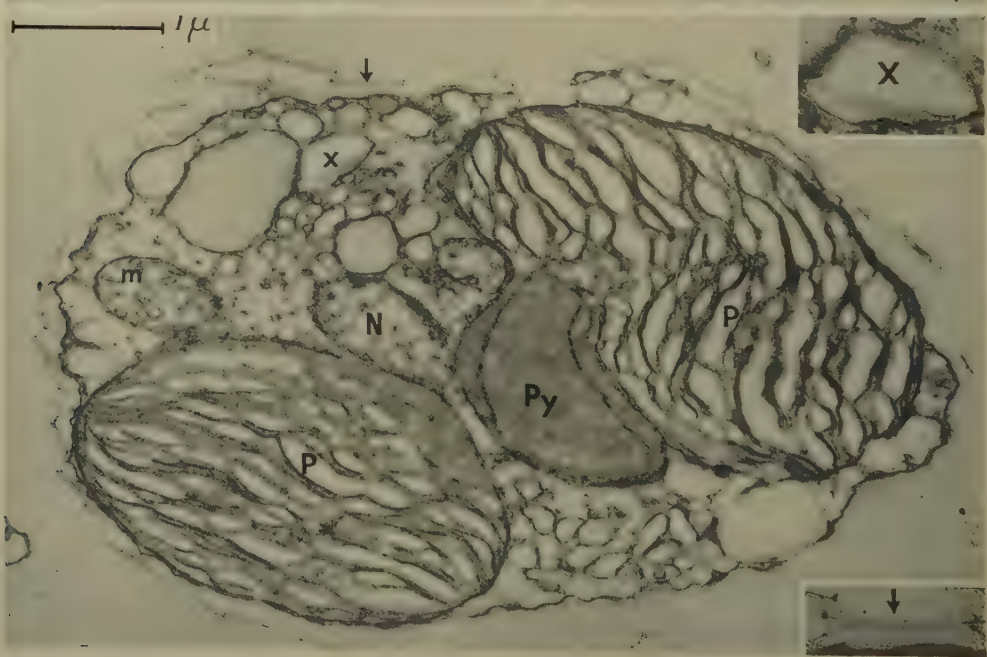
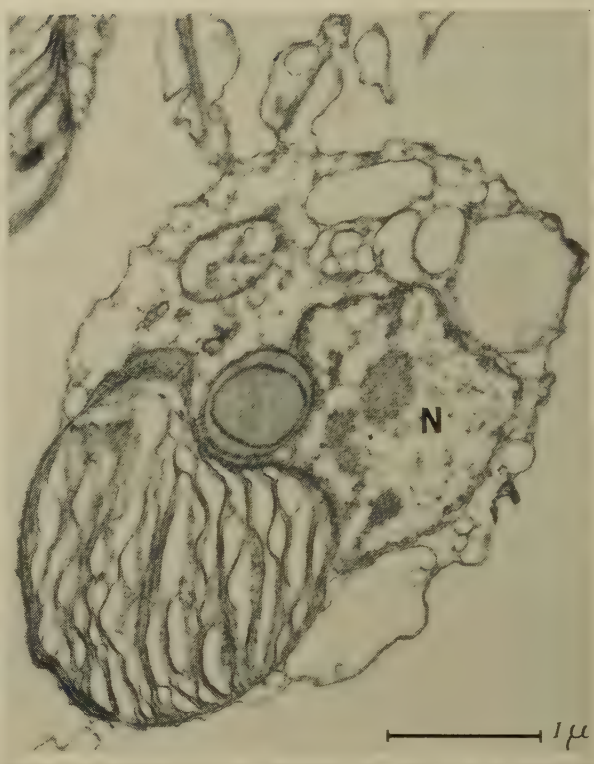
11



12

14
Py
P
N
1 μ
13

15



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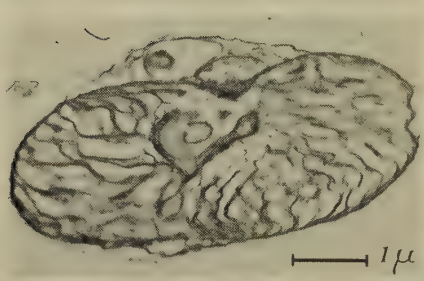

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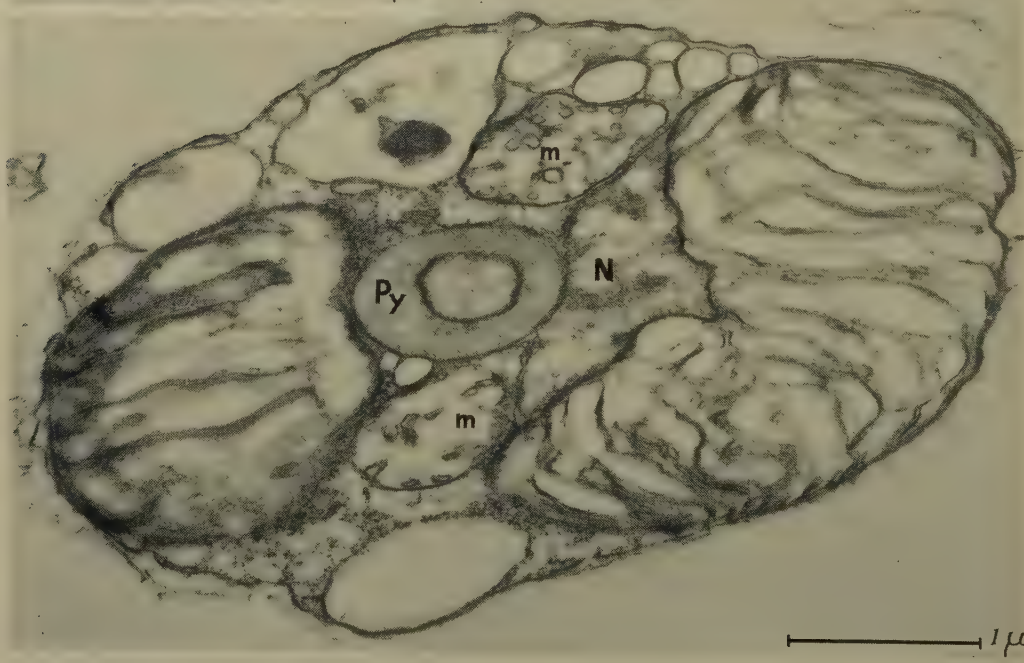

18

cytoplasmic boundary covering the plastid or pyrenoid. There is thus potentially an uninterrupted cavity enveloping the surfaces of both nucleus and plastid. This observation is of interest for the light it throws on the nature of the plastid surface itself. However, the physiological or other importance, if any, of this sharing of potential spaces is entirely unknown.

These facts having been established it was possible to study the living cells with anoptral contrast and to select favourable individuals in which the pyrenoids could be seen. Pl. I, figs. 3–6, illustrate this, while figs. 7–9 show the transformation which occurs when a cell becomes flattened by pressure from the coverslip as liquid from the mount evaporates. At first the pyrenoids appear circular (fig. 7), but as pressure increases they suddenly burst (fig. 8), each leaving a conspicuous bright granule of unknown nature behind.

The haptonema has been found to resemble closely the longer haptonemata of other anatomically known species and can be dealt with briefly. There are the usual three concentric membranes surrounding a ring of seven fibres (or tubes) and it will suffice to reproduce (Pl. III, fig. 14) three sample profiles from one specimen in illustration.

Attention can perhaps usefully be directed to one other detail, illustrated by the inset pictures on Pl. II, fig. 12. In *C. minor* the fine sculpturing on the surfaces of the scales is more easily distinguishable in sections than is usual in the small-scaled species of *Chrysochromulina*, and we have several examples of scales encountered within vesicles, one of which happens to be included in the section in Pl. II, fig. 12. The vesicle (*x*) is shown more highly magnified in the top inset and some superficial scales are reproduced at the same magnification in the bottom inset. Though the complication of phagotrophic feeding should not be forgotten, these vesicles do not look like food vacuoles and are probably interpretable as evidence of internal scale production of the same general type as recently demonstrated more fully for *Micromonas squamata* (by Manton & Parke, 1960) and *Paraphysomonas vestita* (by Manton & Leedale, 1961 *b*).

NEW OBSERVATIONS ON *CHRYSOCHROMULINA KAPPA*

This species proved to be much more difficult to investigate with the methods used here and it was necessary to modify the fixation procedure before satisfactory sections could be obtained. Shortening the fixation time from 1 h to 10 min proved effective, after which it could be seen that the structure is essentially like that of *C. minor* except for the rather exaggerated behaviour of the nucleus.

As in *C. minor* the pyrenoid is attached centrally to the inner face of a plastid though it is less strongly flexed and may be longer. An average width of rather less than 1 μ is associated with a length of up to 2·5 μ. As in *C. minor* the pyrenoid undoubtedly divides with the plastid at each cell division.

From the dimensions and attitude of the pyrenoid when not in division, it might have been anticipated that it would be more conspicuous when viewed in life with anoptral contrast than was found with *C. minor*. This expectation was by no means fulfilled and without the precise information from the electron micrographs as to what to look for in this species, photographic demonstration of pyrenoids in living cells would have been almost impossible. With this information, after prolonged search, several favourable cells were encountered, two of which yielded the photographs for Pl. V, figs. 19 and 20.

The reason for this difficulty lies in the behaviour of the nucleus. This is commonly applied so closely to a pyrenoid as to be virtually wrapped round it, contact with other surfaces of a plastid being less conspicuous. The two sections cut in planes at right angles (Pl. V, figs. 21, 22) are self-explanatory in this respect, though it may be added that fig. 22, is part of a series of sections which at other levels show the pyrenoid as if completely surrounded by the nucleus as is also the case in Pl. VI, fig. 23. In such a condition the circular profile of a transversely cut pyrenoid may look misleadingly like a nucleolus although the delimiting surface membranes unequivocally distinguish it from a true nucleolus which these nuclei also possess. The nature of the 'non-refringent material' which troubled the light-microscopist (cf. p. 520 above) is thus established, and with it the reason for the almost complete invisibility of pyrenoids in a majority of living cells.

As in *C. minor* some observations on haptonema structure have been obtained. In this species, for the first time, a ring of 8 internal fibres or tubes

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Explanation of Plates V, VI

Chrysochromulina kappa

PLATE V

Figs. 19, 20. Two cells photographed alive with anoptral contrast to show pyrenoids in profile (fig. 19) and end on (fig. 20). × 2000.

Fig. 21. Section passing through the base of a haptonema (left) and showing a plastid (*P*) with subtended pyrenoid (*Py*) cut longitudinally and closely invested by the nucleus (*N*), a branched mitochondrion (*m*) above. Micrograph B497, × 20,000.

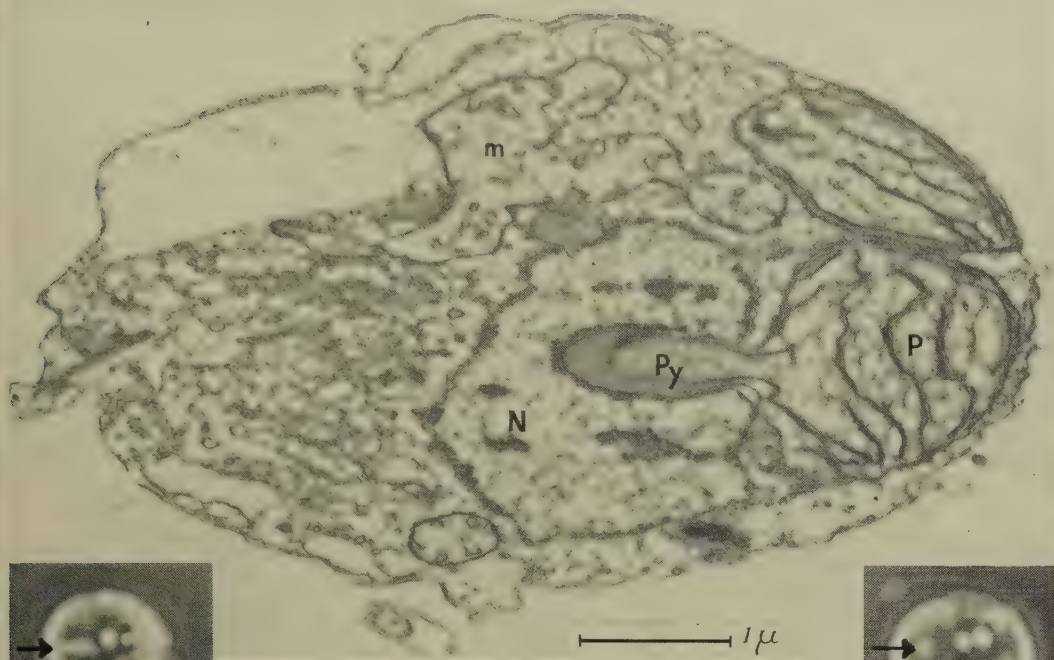
Fig. 22. Another cell showing a transversely cut pyrenoid (*Py*) closely invested by the nucleus (*N*) and with a 'peculiar' Golgi body (*g*), parts of two plastids (*P*) and other inclusions also in the field. Micrograph B486, × 25,000.

PLATE VI

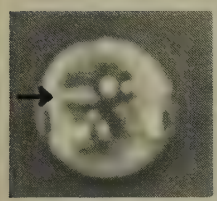
Fig. 23. Cell showing a transversely cut pyrenoid (*Py*) penetrating deeply into the nucleus (*N*) and appearing as if completely surrounded by it (contrast with figs. 21 and 22), mitochondria and part of a Golgi body visible near the base of the flagellum (*F*). Micrograph B1156, × 30,000.

Fig. 24. Four profiles from one haptonema showing a central ring of eight fibres or tubes within the customary three concentric membranes. Micrograph B218, × 60,000.

21

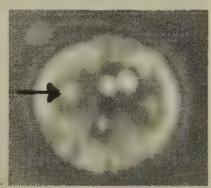


19

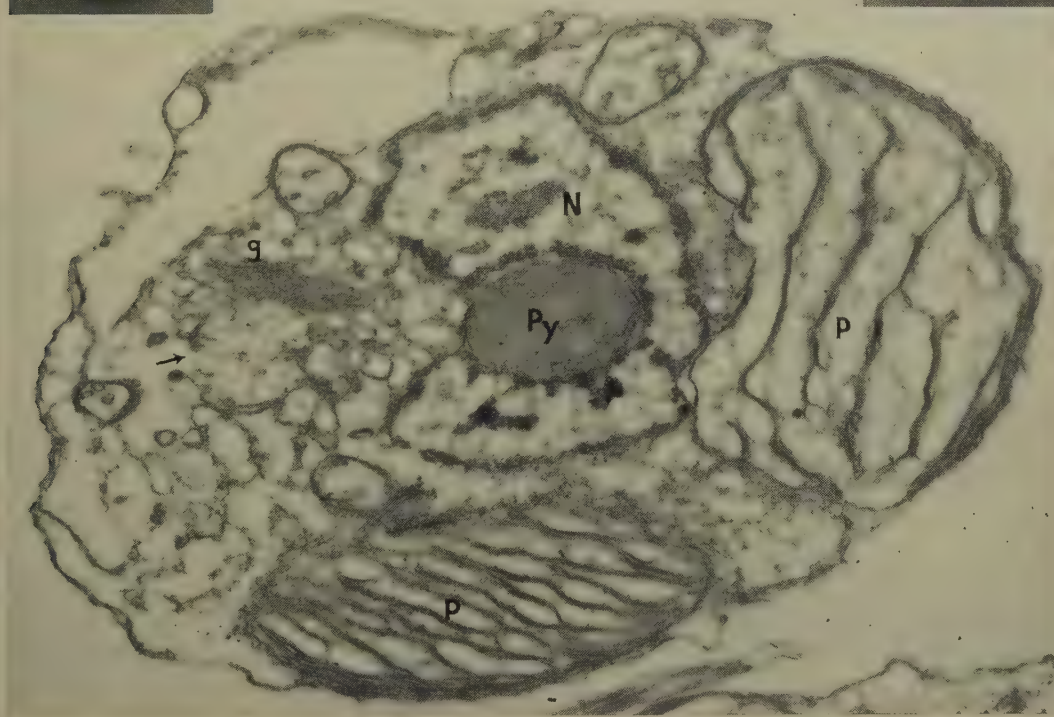


1 μ

1 μ

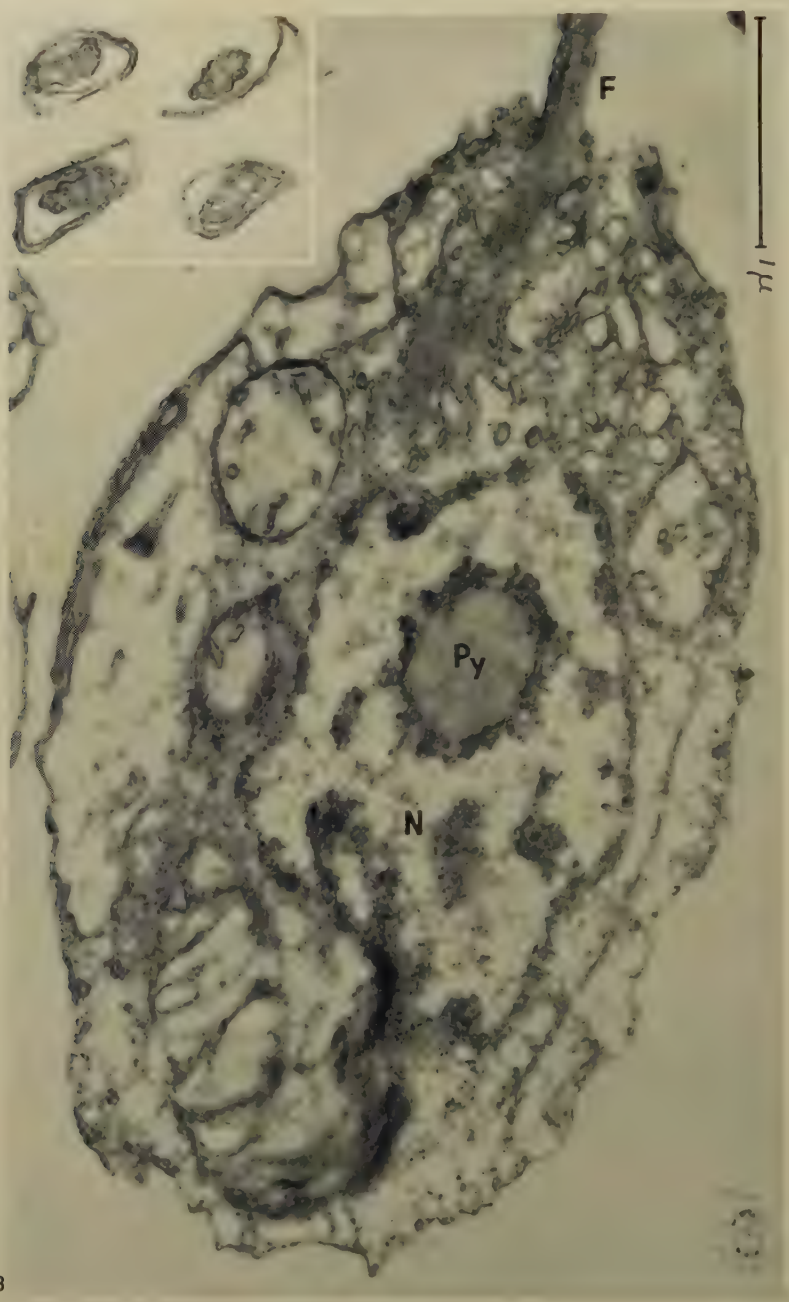


20



22

24



23

has occasionally been found (Pl. VI, fig. 24). This is, however, not universal and the more usual condition of 7 internal fibres or tubes has also been seen.

Pl. V, fig. 22 and Pl. VI, fig. 23, also contain views of Golgi structure to which attention can usefully be directed. As in other cases the single Golgi body (except in dividing cells) is situated close to the bases of the appendages (fig. 23), although the precise significance of this position is still unknown. 'Peculiar' Golgi structure can also be demonstrated (Pl. V, fig. 22) although this feature is much more delicate both in *C. kappa* and *C. minor* than in the other species in which this structure has been found (Parke *et al.* 1959; Manton & Leedale, 1961 *a*). The significance of this component also remains unknown and without the improvement in fixation obtained by the modified treatment it would not have been preserved.

Finally, attention should perhaps be directed to the branched mitochondrion in Pl. V, fig. 21, which may indicate a growth process in some ways comparable to that recently investigated in *C. brevifilum* (see Manton, 1961), and to the base of the haptonema included in fig. 21.

DISCUSSION

It is impossible to determine by any methods at present available whether the special contact established in both these species between the nucleus and the plastid or pyrenoid surface is metabolically significant or not. If an interchange of materials between these organelles could be directly demonstrated the projecting pyrenoid itself might become interpretable in an adaptive sense as in some way facilitating this. On the other hand, the differences found among the various species examined, including these two, somewhat militates against this suggestion. A purely mechanical interpretation of the position of the nucleus seems at least as likely.

In other respects there is clearly a very considerable agreement among all the species attributed to *Chrysochromulina* for which the anatomy is known. These are now eight in number. Projecting pyrenoids occur in *C. chiton*, *C. minor* and *C. kappa*; immersed pyrenoids in *C. strobilus*, *C. erecina*, *C. ephippium*, *C. alifera* and *C. brevifilum*. The haptonema structure is similar in all these species except for minor numerical differences in the central strands; the commonest number is seven central strands, found in all species except *C. strobilus* which has consistently six and *C. kappa* which occasionally has eight. These are the two most important structures which have so far been dealt with anatomically and the only ones with which the present paper has been intimately concerned.

We are not competent to give taxonomic advice on the weight which should be put upon the morphological facts presented here. We have, however, shown why the pyrenoid is difficult to investigate effectively with the light microscope at present and its inconvenience as a major taxonomic criterion is

obvious. The removal from apparent isolation of the condition previously known only in *C. chiton* is nevertheless a positive contribution towards eventual understanding of the group.

SUMMARY

Chrysochromulina minor and *C. kappa* have been re-investigated by means of electron microscopy of thin sections to add details of the microanatomy of pyrenoids and haptonemata, and by anoptral contrast light microscopy to study pyrenoids in living cells. In both species the pyrenoid is in the form of a diverticulum projecting from the centre of the inner face of a plastid and, in *C. minor*, strongly flexed to lie along it. In *C. kappa* the pyrenoid is commonly enveloped by the nucleus which may conceal it entirely from view in life. Some details of the behaviour of the surface membranes of plastids and pyrenoids in relation to that of the nucleus are given. The haptonema structure in both species is shown to be comparable to that of others in which this appendage is much longer, though an occasional variant with eight instead of seven central fibres or tubes has been encountered in *C. kappa* and is demonstrated. The presence of 'peculiar' Golgi structure is reported for both species and demonstrated for *C. kappa*. Some direct evidence indicating an internal origin of scales from vesicles is demonstrated in *C. minor*. Finally a summary is given of salient structural criteria for all the described species attributed to this genus from the marine plankton, the closest agreement as regards pyrenoid structure in the two species under investigation being with *C. chiton*.

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PARKE, M., MANTON, I. & CLARKE, B., 1955. Studies on marine flagellates. II. Three new species of *Chrysochromulina*. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 579–609. 1958. IV. Morphology and microanatomy of a new species of *Chrysochromulina*. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 209–28. 1959. V. Morphology and microanatomy of *Chrysochromulina strobilus* sp.nov. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 169–88.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

BATHAM, E. J., PANTIN, C. F. A. & ROBSON, E. A., 1960. The nerve-net of the sea-anemone *Metridium senile*: the mesenteries and the column. *Quart. J. micr. Sci.*, Vol. 101, pp. 487–510.

The nerve-net has been re-examined after staining with silver and with methylene blue. Methods are described. The neurites run between the epithelium and underlying muscle. Synaptic junctions between nerve-cells and between these, the muscle field and sensory neurites are illustrated. The retractor surface of the mesentery with its powerful muscle is richly innervated. The weak radial muscles are poorly innervated. The distribution of nerve-cells and sense-cells in the mesenteries and column is related to physiological evidence concerning the through-conduction pathways, facilitated and slow contractions, and other aspects of the behaviour of *Metridium*.

C.F.A.P.

BONE, Q., 1960. A note on the innervation of the integument in amphioxus, and its bearing on the mechanism of cutaneous sensibility. *Quart. J. micr. Sci.*, Vol. 101, pp. 371–9.

The dispute upon the nature of the innervation of the skin in amphioxus is reviewed, and it is shown that in addition to a system of free nerve terminals, there are in certain regions of the body, specialized sensory cells, and complex encapsulated nerve endings.

The cells of origin of the free nerve terminals in the skin are discussed, and it is shown that they fall into several morphologically different categories. In the light of this observation, it is suggested that the innervation of the skin of *Amphioxus* does not provide unequivocal evidence for the view that the different cutaneous sensations are determined by different discharge patterns in the *same* fibres of the nerve rami leading from the skin.

Q.B.

BONE, Q., 1960. The central nervous system in amphioxus. *J. comp. Neurol.*, Vol. 115, pp. 27–64.

The organization of the spinal cord of amphioxus is described in detail, and a functional analysis of the system is attempted.

Somatic- and viscero-motor elements are identified, and it is shown that the somatic-motor cells occur in segmental patterns. In both somatic- and viscero-motor systems, single large neurons are found in each segment and it is suggested that these represent the first-formed elements of each system. Cells which are regarded as homologous to the Rohon-Beard cells of craniate embryos are found segmentally in the somatic-sensory system. The arrangement of the whole is compared with the arrangement of the cord in the early ammocoete larva, and it is concluded that the spinal cord of adult *Amphioxus* is comparable to that of the craniate larva, but that, in some ways, it is more primitive than that of any craniate at any stage, particularly in the possession of groups of segmentally arranged somatic-motor cells.

Q.B.

CALDWELL, P. C. & KEYNES, R. D., 1960. The permeability of the squid giant axon to radioactive potassium and chloride ions. *J. Physiol.*, Vol. 154, pp. 177–89.

An appreciable amount of the radioactive potassium taken up by cleaned squid axons during short exposures to ^{42}K sea water appears to be located in a superficial region, possibly the Schwann cell. On transfer to inactive sea water this part of the radioactive potassium exchanges fairly rapidly with inactive potassium, the time constant being of the order of 1 h. Values for the time constant for the remainder of the radioactive potassium, obtained after prolonged soaking, tended towards a value of about 50 h. Time constants of about 50 h were also found for ^{42}K microinjected into the axoplasm and time constants of this order are therefore probably characteristic of potassium which has either penetrated into or been introduced into the axoplasm. The rate of loss of microinjected ^{42}K was greatly increased by stimulation at 50 impulses/sec. The average influx of chloride obtained in studies with ^{36}Cl was 12·6 pmoles/cm^2.sec and there was an extra entry on stimulation of 0·046 pmole/cm^2. impulse. ^{36}Cl microinjected into the axoplasm was lost with a time constant of about 80 h.

P.C.C.

CARLISLE, D. B., 1958. Neurosecretory transport in the pituitary stalk of *Lophius piscatorius*. *Zweites internat. Symp. Neurosekretion, Lund*, 1957, pp. 18–19. Berlin: Springer-Verlag.

A preparation is described in which the long pituitary stalk of this species may be examined and photographed in the living state under the microscope. By this means transport of neurosecretory bodies down the axons from the hypothalamus to the pituitary has been observed and filmed. Action potentials have been recorded also from the stalk, implying that the axons are capable both of neurosecretory transport and of the transmission of nervous impulses.

D.B.C.

CARLISLE, D. B., 1960. Moulting cycles in Crustacea. *Symp. zool. Soc. Lond.*, No. 2, pp. 109–20.

The act of moulting in arthropods must not be considered as an isolated event but as the most spectacular moment in a continuous cycle of metabolic activity. Each moulting cycle is basically like the one before it and many of the stages have now been shown to be under hormonal control. Secretion of the 'moult-inhibiting hormone' by the endocrine complex of the eyestalk promotes the formation of new tissues and prevents the *Y* organ from secreting more than marginal amounts of its hormone. Once this eyestalk hormone ceases to be secreted into the blood stream the *Y* organ releases a single 'dose' of a moult-promoting hormone which initiates the preparations for the moult, which, once under way, are co-ordinated by a moult-accelerating hormone from the *X* organ. The amount of water taken in at the moult itself is regulated by a further eyestalk hormone. Many other hormones act upon particular moults and modify them in some way, acting mainly upon the stage of proecdysis and making the succeeding moult in some way unique: the moult of puberty and the moult at which a female becomes furnished with the brooding characters are examples. In the same way that the originally similar segments of the vertebrate body have been progressively and uniquely modified, in for example the head, so the originally similar moults of arthropods have been modified, till we have the startling difference between the larval and imaginal moults of Lepidoptera.

D.B.C.

CLARK, R. B. & HADERLIE, E. C., 1960. The distribution of *Nephtys cirrosa* and *N. hombergi* on the south-western coasts of England and Wales. *J. Anim. Ecol.*, Vol. 29, pp. 117–47.

Around the south and south-western coasts of England and Wales, *Nephtys cirrosa* occurs in most clean sandy beaches of the open coast, except where wave action is severe and the sand very coarse, while *N. hombergi* lives in muddy sand and is generally found in the lower reaches of estuaries. The occurrence of one or the other species on a beach is correlated with the grade composition of the substratum. *N. hombergi* tolerates up to 3·8% particles larger than 0·25 mm and between 2·2 and 15·9% particles smaller than 0·125 mm. *N. cirrosa* lives in substrata containing up to 50% particles larger than 0·25 mm (or a higher proportion providing the larger grades are not composed of shell fragments), and up to 1·5% particles smaller than 0·125 mm (or more than 1·5% if there is a proportion of coarse particles in the substratum). A few poorly sorted beaches fall within the range of both species and have mixed populations. The distribution of each species, on the beaches where they occur, is largely independent of tidal level and is determined chiefly by the composition of the substratum at different levels.

R.B.C.

COOPER, L. H. N., 1958. A system for international exchange of samples for trace element analysis of ocean water. *J. mar. Res.*, Vol. 17, pp. 128–32.

For some elements in sea water concentrations reported by different analysts vary by several orders of magnitude. This variability is due at least in part to faulty sampling and storage of samples. Some sources of error are listed.

It is recommended that a few standard oceanic stations should be selected widely spaced around the world and near to laboratories devoted to chemical oceanography. Sampling at these stations should be directed on board by chemists skilled in trace analysis. Work at one such standard station in the Bay of Biscay is described. Proposals are made for a system for international exchange of samples collected in this way.

The matter was discussed informally by the Committee on Chemical Oceanography during the meeting of the International Association of Physical Oceanography in July–August 1960 at Helsinki. Some duplicated copies were made available but in spite of the nominal issue date, 28 November 1958, the printed account had been seen by no one present. No recommendations could therefore be made.

Initiative therefore now rests with analysts who would like to see the recommendations implemented.

L.H.N.C.

CURRY, DENNIS, 1960. Eocene limestones to the west of Jersey. *Geol. Mag.*, Vol. 97, pp. 289–98.

This note records the presence of Eocene marine limestones *in situ* on the bed of the English Channel along an east–west line to the west of the island of Jersey. The microfauna of samples of these limestones is analysed and is held to indicate that they are of Lutetian or Auversian age. It is noted that limestones of similar age and lithology occur near Valognes in the Cotentin. There are comments on the relevance of the above discoveries to the structural history of the area.

D.C.

HUGHES, G. M., 1960. The mechanism of gill ventilation in the dogfish and skate. *J. exp. Biol.*, Vol. 37, pp. 11–27.

The respiratory movements of *Scyliorhinus canicula* and *Raia clavata* were studied using cinematography and electrical recording methods. In both species movements of the mouth precede those of the spiracular valve and of the branchial region. In *Scyliorhinus*, expansion and contraction of the gill pouches spreads from the first to the last pouch but in *Raia* these movements are almost synchronous. Water entering one side of the mouth leaves by the three posterior gill slits of the same side. Water entering the spiracle leaves through the anterior slits of the same side. This separation of flow is less marked in *Raia* where a relatively greater volume enters through the spiracle.

Pressure curves recorded by fine hypodermic needles placed inside the mouth spiracle and gill slits all show phases which are positive and negative with respect to the external medium. The precise form of these curves indicates that the passage of water across the gill filaments is because (i) contraction of the oral-branchial cavity forces it through and (ii) it is also sucked through by the expansion of the cavities (parabranchial) external to the gills.

The flaps over the gill slits prevent the entry of water to the latter. These valves are passive in the dogfish but it seems probable that they are actively controlled in *Raia*. Observations have also been made which suggest that dogfish and sharks do not make respiratory movements during fast swimming although they immediately commence active ventilation on coming to rest.

G.M.H.

HUGHES, G. M., 1960. A comparative study of gill ventilation in marine teleosts. *J. exp. Biol.*, Vol. 37, pp. 28–45.

Movements of the lower jaw and operculum were recorded simultaneously with the associated pressure changes in the buccal and opercular cavities during breathing of the following species—*Trachurus trachurus* (L.), *Clupea harengus* L., *Gadus merlangus* L., *Onos mustela* (L.), *Crenilabrus melops* (L.), *Cottus bubalis* Euphrasen, *Blennius ocellaris* L., *Trigla gurnardus* L., *Callionymus lyra* L., *Pleuronectes platessa* L., *Microstomus kitt* (Walbaum), *Conger conger* (L.), *Syngnathus acus* L.

In all species ventilation is achieved by the action of a buccal pressure pump and opercular suction pumps, but there are differences in the relative importance of these two pumps which are related to the habitat of the fish. The suction pump is of greater importance in those fishes which spend most of their lives on the sea bottom. In several species the differential pressure curve does not include a brief period of reversal in pressure gradient which has been found in most fishes so far investigated. Notable among these species are the two flat fishes investigated, and in these there is some evidence for active control of the opercular valves.

G.M.H.

KENNEDY, G. Y. & NICOL, J. A. C., 1959. Pigments of *Chaetopterus variopedatus* (Polychaeta). *Proc. roy. Soc.*, Ser. B, Vol. 150, pp. 509–38.

The intestine in the middle and posterior regions of *Chaetopterus variopedatus* is green. The coloration is produced by phaeophorbides *a* and *b*, the former predominating. Other pigments present in the gut are the chlorophyll-derivatives isophaeophorbide *d*, dioxymesophyllochlorin, copper phaeophorbide chelation compounds, coproporphyrin III, bile-pigment type compounds, turbo-glaucobilin and helioporobilin, and carotenoids, β-carotene and xanthophyll. The body wall contains

β-carotene. Melanin occurs in the black chaetae, and a reddish melanoid pigment in the head. Phaeophorbides *a* and *b* are derived from chlorophylls *a* and *b* in the animal's food. Phaeophorbides are contained within green spherules in cells of the gut wall. These spherules are not symbionts. They are a constant feature of the animal, even during prolonged starvation, and appear to play some essential biochemical role.

J.A.C.N.

LEBOUR, M. V., 1959. The larval decapod Crustacea of Tropical West Africa. *Atlantide Rep.*, Vol. 5, pp. 119–43.

This is a brief survey of the decapod larvae obtained by the Danish Atlantide Expedition. Although a large number of the species cannot be determined, the more important are placed, as nearly as possible, in their genera. There are many interesting deep water forms—sergestids, penaeids and carids. Among the Caridea are many *Eretmocaris* larvae, one of which is almost certainly *Lysmata moorei*, common in the district but whose larva was not known. The larva of *Latreutes parvula* is also described for the first time.

The Hippidae are well represented and there is a new form of *Albunea* larva, unlike any so far known. *Hippia cubensis* larva is common and not previously recognized. A number of *Dorippe* larvae occur amongst which is *Dorippe armata*, identified from a number of megalopae emerging from the larvae.

The report is illustrated by twenty-one line drawings and a list of larvae and the various stations is given.

M.V.L.

MURRAY, R. W. & POTTS, W. T. W., 1961. The composition of the endolymph, perilymph and other body fluids of elasmobranchs. *Comp. Biochem. Physiol.*, Vol. 2, pp. 65–75.

Serum, cranial fluid, perilymph, endolymph and jelly from the ampullae of Lorenzini of *Raja* have been analysed for Na, K, Cl and urea. The concentrations in mM/kg water are: perilymph Na 281, K 3·54, Cl 321, urea 447; endolymph Na 295, K 63·4, Cl 391, urea 381; Lorenzini jelly Na 443, K 12·5, Cl 581, urea 74·8. Serum and cranial fluid are similar to perilymph. The endolymph has 19 times as much potassium as the perilymph. The composition of the Lorenzini jelly is unlike that of any other fluid. Freezing-point depressions show that the Lorenzini jelly is 5% hypertonic to sea water, and the other fluids are all approximately 2% hypertonic.

R.W.M.

RUSSELL, F. E. & EMERY, J. A., 1960. Venom of the weevers *Trachinus draco* and *Trachinus vipera*. *Ann. N.Y. Acad. Sci.*, Vol. 90, pp. 805–19.

The venom apparatus of these fishes consist of two opercular spines, five to eight dorsal spines, and the tissues contained within the integumentary sheaths surrounding the spines. Some of the physical and chemical properties of the venom are discussed. The venom has no remarkable effect on the neuromuscular junction, but does exert a deleterious effect on the cardiovascular system. It produces a precipitous fall in systemic arterial pressure concomitant with changes in the pulse pressure, cardiac rate, pulmonary arterial pressure, pulmonary artery flow, venous and cisternal pressures, respiration, and provokes changes in the electrocardiogram and electroencephalogram. The principal action of the venom appears to be on the heart muscle.

F.E.R.

SUTTON, M. F., 1960. The sexual development of *Salpa fusiformis* (Cuvier). Part I. *J. Embryol. exp. Morphol.*, Vol. 8, pp. 268–90.

The early sexual development of *Salpa fusiformis* (Cuvier) is described. The fertilized egg begins to develop in the single ovarian follicle, dorsolaterally, close to the atrial epithelium. At the eight-cell stage the follicle cells (calymmocytes) proliferate rapidly and insinuate themselves between the blastomeres. Each blastomere at this point buds endogenously and the resultant seven daughter cells rapidly degenerate; a large residual cell persists. The calymmocytes continue to increase in number but their degeneration also is initiated. The follicle perforates the atrial epithelium, and is now known as the blastophore. A normal blastomere division occurs and is followed by a second phase of budding and daughter-cell degeneration. A structure, the placental knob, develops ventrally; one of its functions is to proliferate calymmocytes. The blastomeres begin to move around regularly in irregularly occurring spaces produced by calymmocyte degeneration, and, on gaining contact with each other, normal division is resumed. A medio-dorsal, neuro-chordal cell massif becomes recognizable and subsequently it moves antero-dorsally. Three regularly occurring cavities begin to develop during the neuro-chordal cell movement. They are centrally and laterally placed and will ultimately fuse and form, in part, the oozooid pharynx. The remaining blastomeres aggregate in groups which will give rise to the cloacal epithelium, remaining ectoderm, and endoderm. Mesodermal cells are not yet to be discerned with certainty. During the early stages of development of the three cavities an incubation fold develops from the adjacent atrial epithelium. It grows up around the blastophore. The aggregation of the cloacal, ectodermal, and endodermal blastomeres and the fusion of the central and lateral cavities occur after the closure of the fold.

The significance of certain phenomena, in particular of endogenous budding, is discussed.

M.F.S.

WILLIAMS, H. HARFORD, 1960. A list of parasitic worms, including twenty-two new records, from marine fishes caught off the British Isles. *Ann. Mag. nat. Hist.*, Ser. 13, Vol. 2, pp. 705–15.

In a list of 84 species of helminth parasites 14 cestodes of the order Tetraphyllidea and one of the Tetrarhynchidea were recorded from elasmobranchs caught in the Plymouth area. *Crossobothrium angustum*, *Anthobothrium laciniatum*, *Prosobothrium armigerum* and *Platybothrium auriculatum* were new records for the British Isles.

H.H.W.

WILLIAMS, H. HARFORD, 1960. The intestine in members of the genus *Raja* and host-specificity in the Tetraphyllidea. *Nature, Lond.*, Vol. 188, pp. 514–16.

Differences in the structure of the mucosa of the intestine in some species of the genus *Raja* were described. In *R. montagui* the mucosa is composed of variously arranged and folded ridges which join to form a complicated network giving the surface of the intestine a corrugated and an irregular appearance. In *R. clavata* the mucosa is made up of a fine regular network of ridges the intervening spaces leading into tubular crypts and the surface is relatively smooth. In *R. naevus* well developed villi are present. In their general appearance the intestines of *R. brachyura* and *R. microocellata* resemble those of *R. montagui* and *R. clavata* respectively, but there are

differences in their detailed structure. It was suggested that the above differences may be of taxonomic value, that rays may be more selective in their feeding habits than hitherto believed and that a correlation may occur between their feeding habits and the structure of the intestine. It was stated that since a correlation occurs between the morphology of the intestine in elasmobranchs and the structure of the scolex of their cestodes this should be considered in studies on the host-specificity of the Tetraphyllidea.

H.H.W.

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COMPARISON BETWEEN THREE METHODS OF MEASURING UNDERWATER ILLUMINATION IN COASTAL WATERS

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The Plymouth Laboratory

(Text-fig. 1)

Two investigations are here combined which developed *ad hoc* at the end of the Second World War and shortly afterwards. The first compared extinction coefficients of sea water obtained by an aerial photographic method (Moore, 1947) with those obtained on water samples in a Pulfrich photometer. The second compared measurements with the Pulfrich photometer with measurements by the late Dr W. R. G. Atkins in the sea with photoelectric cells. The intention was to achieve a correlation between the aerial photographic technique of Moore and the photoelectric cell methods of Atkins and Poole using the Pulfrich photometer as a bridge.

There were faults in the design of both investigations but the repetition by more modern techniques, felt to be desirable, has never been achieved; and now that Dr Atkins has died, it is quite clear it never will be. The results, imperfect though they are, seem to be of sufficient interest to justify publication of the manuscript prepared 13 years ago.

The Zeiss Pulfrich Photometer was used (Kalle, 1938; Cooper & Milne, 1938, 1939) for measuring extinction coefficients of light in sea waters in which scattering occurs. Results have now been found to be two to five times greater than those obtained by other methods. The discrepancies are due to forward scattering of light and variations in the geometry of the light paths in the several methods (see Appendix, p. 548).

COMPARISON WITH MOORE'S BRIGHTNESS PROFILE METHOD

Moore (1947) described his method as follows: if a sandy beach is photographed vertically from the air through a colour filter, its apparent brightness is found to vary in a simple way with the depth and clarity of the water over it; if the clarity (or the extinction coefficient) of the water is known, the depth of water can be determined by measuring the relative brightness at different points on a single air photograph. If it is not known, the measurements may be made on a pair of photographs taken simultaneously through two special colour filters; since a relationship between the two extinction coefficients may

be assumed, depths can be determined from the photographs alone without any other source of information. The method proved highly consistent but during development a cross-check against another device, the Pulfrich photometer, was sought. On nine occasions in August 1945 water sampling was synchronized with the aerial photography. The bays studied are a few miles east of Falmouth. The weather was perfect.

Moore's results (1947, tables 7 and 8) have been multiplied by 7·55 to convert to extinction coefficients based on Napierian logarithms and the metre as unit of length.

Experimental

The instrument

The method of measurement in the Pulfrich photometer using 25 cm tubes has been fully described (Cooper & Milne, 1938), but to obtain significant readings from the English Channel waters tubes 1·00 m long and 4·0 cm internal diameter had to be made.

The Zeiss assembly of eyepiece, spectral filter holders and filters, logarithmic sectors and drums was used without modification. An optical bench to carry the measuring assembly, absorption tubes and the Zeiss lamp-housing in the blacked-out cabin of the motor launch 'Vita' was built to an exacting specification.

Distilled water was prepared at Plymouth and used at sea within a week.

Procedure. Due to slight faults in the hastily made metre tubes, standard Pulfrich procedure was impracticable. One tube filled with distilled water was used as a dummy always, and only on the left-hand side of the instrument. It was marked and set always in the same position. The second tube had to be marked and used similarly on the right-hand side. At the start of the day's work the second tube was also filled with distilled water and matched four times with each spectral filter against the dummy tube. The left-hand drum had to be set at such a reading that the right-hand drum gave a transmission D around 40–50 %. Let this right-hand drum reading be a.

The right-hand tube was then emptied and twice rinsed with the water to be examined. Next it was filled with the sample taking care that no air bubbles became entrapped. This sample was then matched four times with each spectral filter against the dummy, the adjustment being made with the *right*-hand drum. Let the mean reading be b which was always greater than a.

Then

$$\mu_M = \log \frac{I_{H_2O}}{I_{SW}} = \log b - \log a. \qquad (1)$$

If an unexpectedly turbid water appeared requiring a right-hand drum reading in excess of 100, the limit of the scale, it was necessary only to reduce the arbitrary left-hand drum reading from g_1 to g_2 sufficiently to bring the right-hand drum reading within the range of the scale. The correction term

$$\log g_1 - \log g_2$$

had then to be added to obtain μ_M.

With these precautions it is believed that the results obtained were little inferior to those that would have been obtained with perfect tubes used in the conventional way.

Correction term, μ_{λ, H_2O}

In many marine problems one needs to know how much light of a given colour reaches a given depth. It is immaterial whether the light has been absorbed or scattered by water substance, by materials in solution or materials in suspension. It is a grave disadvantage of all methods which compare a sample of a natural water with pure water that the absorption by water substance is not included. In the middle of the visible spectrum the uncertainty may not be of much consequence, but at the red end it may be important. In the red the assessment of the large correction term is intractable. It is now very clear that laboratory absorptiometers involving comparison with distilled water should never be used where a submarine photometric or brightness profile technique is available and appropriate. None the less a detailed examination of how the correction may be assessed may be helpful.

For monochromatic light

$$\mu_{\lambda, SW} = \mu_{\lambda, M} + \mu_{\lambda, H_2O}. \tag{2}$$

where the symbols and those below have the meaning described in the glossary of terms (p. 550). Since the spectral filters select a wave-band between wave-lengths λ_1 and λ_2, a mean correction factor $\mu_{\text{mean}\,\lambda, H_2O}$ is needed. It depends on the length of the absorption tube and may be computed by the formula of Cooper & Milne (1939):

$$\mu_{\text{mean}\,\lambda, H_2O} = 2{\cdot}303 \log_{10} \frac{\int_{\lambda_1}^{\lambda_2} c_\lambda \mathcal{J}_\lambda V_\lambda \, d\lambda}{\int_{\lambda_1}^{\lambda_2} c_\lambda \mathcal{J}_\lambda V_\lambda \exp(\mu_\lambda l) \, d\lambda} \tag{3}$$

For $\mathcal{J}_\lambda$ the monochromatic extinction coefficients determined for pure water by James with Birge (1938) were used and the two integrals evaluated graphically, in order to determine $\mu_{\text{mean}\,\lambda, H_2O}$:

Length of absorption tube	Spectral filter							
	S43	S47	S50	S53	S57	S61	S66	S72
1 m	0·008	0·006	0·007	0·025	0·078	0·28	0·41	1·58
25 cm	0·008	0·006	0·007	0·025	0·078	0·27	0·41	1·63

For the filters S43–S61 the accuracy of these correction terms is better than the experimental error. For the red filters, due to the characteristics of the photometer lamp, the spectral filters and the human eye, the error of the correction term is greater and its order of magnitude may be computed. Thus for coastal sea water, such as that examined in this work, the standard error of measurement with the red S66 filter amounts to about 0·005–0·01 and with the deep red S72 to 0·02–0·09.

Sampling at sea. At Portscatho on 2 August distances of the launch from shore were established by Army shore surveyors. On other occasions distances were estimated by eye and later checked from Moore's aerial photographs. Bottom depths are accurate to ±3 in. Tide is given as local high water. All samples were taken with a 1·7 l. Nansen–Pettersson water-bottle hauled by hand. Usually samples were run into the absorption tube and examined at once but some had to be stored in glass vessels for as much as 3 h. These were well swirled but not shaken before transfer. Measurements were made as quickly as possible ending always with a repeat with the first filter used. Station data are set out in Table 1.

Comparison of extinction coefficients determined at Portscatho

In Table 2 will be found extinction coefficients μ_M measured against distilled water. To obtain the extinction coefficients for sea water μ_{SW}, found with each Pulfrich filter, the coefficients for distilled water μ_{H_2O} given at the foot of Table 2 have to be added as a correction term. Moore used coefficients corrected in this way by the writer.

It is debatable at what stage the distilled water correction is best applied. The writer now prefers to apply it *after* the transformation of the Pulfrich results μ_M in terms of Moore's Wratten filters. Moore (1947, table 3) for a number of wave-lengths gives the product $Z_\lambda\, Y_\lambda$ of the relative sensitivity of Kodak aero Super XX film to mean noon sunlight Y_λ and the transmission of the Wratten filters Z_λ. This serves the same purpose as the expression $c_\lambda \mathcal{J}_\lambda V_\lambda$ of Cooper & Milne (1939, p. 292) (see glossary of terms).

TABLE 1. STATION DATA

Position of motor launch 'Vita'	Serial number of water sample	Depth in feet: Bottom	Depth in feet: Sample	Temperature (°C)	Time of sampling of water (G.M.T.)	Time of aerial photograph (G.M.T.	Serial number of negative
Series 2. Portscatho. 2 August 1945. Flat calm. Local high water 11.36 h G.M.T.							
Anchored 675 ft. from sea wall (fixed by shore surveyors)	3	16¾	1	17·73	14.13	14.44	0900
	4	16¾	15	16·38	14.45	—	—
Anchored 3070 ft. from sea wall (fixed by shore surveyors)	5	78	1	16·83	10.23	10.29	0801
	6	78	25	16·30	11.14	—	—
	7	78	70	15·17	10.56	—	—
Return visit to 3070 ft. station	8	—	15	16·31	15.53	—	—
	9	—	20	16·12	15.44	—	—
	10	—	25	15·90	15.32	—	—
Series 3. Porth Farm. 3 August 1945. Flat calm. Local high water 12.49 h G.M.T.							
Anchored 300 ft. estimated from shore and 50 ft. east of surveyed line	11	9⅙	4	16·40	09.52	09.50	1134
Anchored on survey line	12	28	1	17·3	11.16	11.07	1170
	13	28	10	—	11.04	—	—
	14	28	25	—	10.47	—	—
Series 4. Portscatho. 3 August 1945. Flat calm. Local high water 12.49 h G.M.T.							
Anchored on surveyed line. Position shown by Moore (1947, plates 11 and 12)	15	56	1	18·09	13.48	—	—
	16	56	1	18·82	15.04	—	—
	17	56	5	16·79	14.33	14.20	975
	18	56	19	16·08	14.20	14.21	976
	19	56	25	16·08	13.29	14.23	979
Lying 200–250 ft. off shore. Tide falling rapidly. Depth under 'Vita' 7½ ft. at 15.35 h, 5 ft. 10 in. at 16.21 h. All samples taken from dinghy	20	2½	1	—	15.40	15.35	1008
	21	6	2½	—	15.52	15.37	1012
	22	8	3¼	—	16.30	—	—

The calculation consists of two parts: first, to compute the extinction coefficient μ_M which would be observed in our modified Pulfrich photometer if (*a*) the Zeiss spectral filters were replaced by the Wratten filters used in the aerial camera and if (*b*) the electric lamp were replaced by mean noon sunlight; secondly, to compute the correction which such a combination would need for the absorption by water substance in the twin tube.

Inspection of the appropriate $c_\lambda \mathcal{J}_\lambda V_\lambda$ and $Z_\lambda Y_\lambda$ curves suggested that the extinction coefficient μ_M appropriate for the Wratten green filter with mean noon sunlight could be built up from the Pulfrich μ_M measurements as $\frac{1}{2}(\frac{2}{3}\mu_{S50}+\mu_{S53}+\frac{1}{3}\mu_{S57})$ where μ_{S50}, etc., represent the extinction coefficients measured with the Zeiss Pulfrich S 50 filter, etc. Since the simple arithmetic mean, $\frac{1}{3}(\mu_{S50}+\mu_{S53}+\mu_{S57})$, differed only slightly from this (mean difference 0·004, maximum differences 0·014 and 0·009 on determinations exceeding 0·27) a more exact but very tedious calculation was not justified.

TABLE 2. MEASURED EXTINCTION COEFFICIENTS (μ_M) WITH PULFRICH PHOTOMETER IN 1 M TUBES

Serial number of water sample	Depth sampled in feet	Measured extinction coefficient μ_M with Pulfrich spectral filter number							
		S 43 violet	S 47 blue	S 50 blue-green	S 53 green	S 57 yellow	S 61 orange-red	S 66 red	S 72 deep red
		Series 2. Portscatho. 2 August							
3	1	0·50	0·40	0·39	0·29	0·29	0·27	0·23	0·23
4	15	0·59	0·55	0·45	0·43	0·35	0·36	0·35	0·31
5	1	0·38	0·33	0·31	0·26	0·25	0·24	0·21	0·19
6	25	1·10	0·91	0·76	0·58	0·56	0·54	0·53	0·55
7	70	0·83	0·74	0·67	0·64	0·60	0·57	0·58	0·54
8	15	0·40	0·37	0·35	0·29	0·32	0·30	0·29	0·26
9	20	0·53	0·46	—	0·40	—	0·39	—	—
10	25	—	0·43	—	0·37	—	0·35	—	—
		Series 3. Porth Farm. 3 August							
11	4	0·49	0·39	0·30	0·30	0·25	0·24	0·25	0·25
12	1	0·48	0·36	0·30	0·30	0·24	0·26	0·24	0·21
13	10	0·40	0·35	0·27	0·27	0·24	0·25	0·21	0·24
14	25	0·43	0·36	0·30	0·31	0·30	0·28	0·24	0·20
		Series 4. Portscatho. 3 August							
15	1	0·31	0·29	0·24	0·24	0·23	0·21	0·21	0·15
16	1	0·46	0·31	0·22	0·23	0·21	0·23	0·20	0·16
17	5	—	0·29	—	0·26	—	0·27	—	—
18	19	—	0·35	—	0·33	—	0·32	—	0·26
19	25	0·39	0·38	0·31	0·32	0·27	0·32	0·31	0·29
20	1	1·00	0·87	0·81	0·77	0·74	0·70	0·62	0·64
21	2½	0·51	0·41	0·34	0·31	0·31	0·26	0·38	—
22	3¼	0·51	0·46	0·36	0·37	0·34	0·32	0·26	—
To obtain extinction coefficient in sea water μ_{SW} add μ_{H_2O} =		$0{\cdot}00_8$	$0{\cdot}00_6$	$0{\cdot}00_7$	$0{\cdot}02_4$	$0{\cdot}07_8$	$0{\cdot}28_0$	0·41	1·58

At intervals of 20 mμ the product of $Z_\lambda Y_\lambda$ (see above) and μ_{H_2O} for monochromatic light as given by James with Birge (1938) was evaluated. Integration gives the extinction coefficients of pure water μ_{H_2O} which would occur in the modified Pulfrich photometer equipped with the Wratten filters in mean noon sunlight. The correction term is 0·05 for the Wratten green filter and 0·29 for the Wratten red filter.

TABLE 3. EXTINCTION COEFFICIENTS FOR GREEN AND RED LIGHT DETERMINED WITH WRATTEN FILTERS

(*a*) Cross-computed from measurements (Table 2) with the spectral filters of the Pulfrich photometer and corrected for absorption by water substance (columns 3 and 4).

(*b*) Determined directly by the brightness profile method (columns 7 and 8).

(*c*) Ratios between extinction coefficient determined by the Pulfrich photometer and by the brightness profile method (columns 9 and 10).

Determinations based on the Pulfrich photometer				Determinations based on brightness profiles				Ratio of coefficients	
Serial number of water sample	Depth sampled in feet	Extinction coefficients μ_{SW} No. 56 green	Extinction coefficients μ_{SW} No. 27 red	Serial number of negative	Depth range in feet	Extinction coefficients μ_{SW} No. 56 green	Extinction coefficients μ_{SW} No. 27 red	col. 3 / col. 7 No. 56 green	col. 4 / col. 8 No. 27 red
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
				Series 2. Portscatho. 2 August					
3	1	0·37	0·54	—	—	—	—	—	—
4	15	0·47	0·64	—	—	—	—	—	—
Mean		0·42	0·59	0900	2–12	0·20	0·42	2·1	1·4
5	1	0·32	0·41	0801	10–24	0·18	0·40	1·8	1·0
6	25	0·68	0·83	—	—	—	—	—	—
7	70	0·69	0·86	—	—	—	—	—	—
				Series 3. Porth Farm. 3 August					
11	4	0·34	0·53	1134	7–21	0·17	0·39	1·9	1·4
12	1	0·34	0·54	—	—	—	—	—	—
13	10	0·31	0·52	—	—	—	—	—	—
14	25	0·35	0·55	—	—	—	—	—	—
Mean of 12–14		0·33	0·54	1170	4–22	0·18	0·41	1·8	1·3
				Series 4. Portscatho. 3 August					
15	1	0·28	0·50	—	—	—	—	—	—
16	1	0·27	0·51	—	—	—	—	—	—
17	5	0·30	0·55	975	4–24	0·18	0·40	—	—
18	19	0·36	0·61	976	2–12	0·20	0·42	—	—
19	25	0·35	0·60	979	2–16	0·16	0·39	—	—
Weighted mean		0·32	0·56	Mean		0·18	0·40	1·8	1·4
20	1	0·82	0·95	—	—	—	—	—	—
21	2½	0·37	0·56	1008	4–18	0·25	0·48	—	—
22	3¼	0·41	0·58	1012	2–16	0·20	0·42	—	—
Mean of 21 and 22		0·39	0·57	Mean		0·23	0·45	1·7	1·3
						Mean		1·8	1·3

Addition of these two quantities gives the extinction coefficient μ_{SW} which should be measured by an instrument of our modified Pulfrich type lit by mean noon sunlight, using the Wratten filter in question and replacing the eye by the aerial camera and its photographic film (Table 3, columns 3 and 4).

Results of the two methods of measurement are compared in Table 3, the gist of which lies in columns 9 and 10 giving the ratios of (*a*) extinction coefficients for mean noon sunlight transmitted by the Wratten filters and recorded by the photographic film as estimated from the Pulfrich measurements to (*b*) coefficients determined directly by the brightness profile method. The ratios are variable, considerably exceed unity and are larger for the green ($\cong$ 1·8) than for the red filter ($\cong$ 1·3). The explanation in terms of forward scattering (Atkins & Poole, 1952) and the geometrical optics of the Pulfrich system has now become clear (see Appendix).

COMPARISON WITH POOLE AND ATKINS'S SUBMARINE PHOTOELECTRIC METHOD

In the English Channel or Plymouth Sound in 1946 Dr W. R. G. Atkins on boad R.V. 'Sabella', immediately before or after his submarine photoelectric measurements, took water samples with the Nansen–Pettersson water-bottle. These were brought back to the laboratory in hydrographic green-glass sample-bottles, about six being needed for one examination. The same night they were examined in the Pulfrich assembly that had been used off Falmouth in 1945 (Table 4, in which the distilled water correction μ_{H_2O} has been added to the extinction coefficients measured, μ_M, to convert them to a basis applicable in the sea, namely $\mu_{SW,P}$).

The Corning blue filter used by Atkins has maximum transmission at about 430 mμ so that results with it are best compared with the Pulfrich violet filter S43, maximum transmission at 434 mμ.

The BG12 filter, also blue, has maximum transmission at 450 mμ and has been compared with the mean of the coefficients found with the Pulfrich filters S43 (434 mμ) and S47 (463 mμ).

The Corning green and VG9 submarine filters and the Pulfrich S53 filter all have maximum transmission close to 530 mμ and admit of straightforward comparison.

The combination OG2+VG9 has a maximum transmission close to that of the yellow S57 filter.

All the red filters have a sharp cut-off in transmission on the side of shorter wave-length but high transmission extends into the infra-red. In the red the extinction coefficient of water substance increases rapidly with wave-length whilst the sensitivity of the human eye and of the several photocells decreases. The Weston selenium cell becomes insensitive above 680 mμ. Moreover the composition of red light in the sea changes very rapidly with depth. The very tedious calculations necessary to allow accurately for these

conflicting sources of error have not been attempted. It has been considered sufficient to compare the submarine measurements made with filter RG1 with Pulfrich filter S61 and those with the Corning red and RG5 filters with Pulfrich filter S66.

Station data

12 *July* 1946. Station in English Channel, 2 miles east of the Eddystone (50° 11′ N., 4° 13′ W.). Clear sun. Cubical submarine photometer. The two methods agree that transparency decreased with depth but do not agree in detail. Whereas for both blue and green light the Pulfrich method showed the maximum decrease in transparency between 20 and 25 m (Table 4), the cubical photometer showed it between 15 and 20 m. The observations on this date are the most satisfactory for purposes of comparison and most weight should be set on them.

26 *August* 1946. International Hydrographic Station E1 (50° 04′ N., 4° 22′ W.). On this occasion the most complete set of submarine photometric measurements are available. Between noon and 15.30 h five different filter combinations were used with the Weston selenium cell which has maximum sensitivity at 580 mμ and is insensitive above 680 mμ. At 15.45 h water samples for Pulfrich examination were drawn from three depths only, namely 0, 5 and 50 m. From Table 4 it will be seen that the Pulfrich extinction coefficients on surface water were 0·07–0·14 greater than those at

TABLE 4. EXTINCTION COEFFICIENTS ($\mu_{SW,P}$) IN WATER FROM THE ENGLISH CHANNEL NEAR PLYMOUTH MEASURED WITH THE PULFRICH PHOTOMETER.

Distilled water correction applied.

Depth (m)	Temp. (°C)	Spectral filter S43	S47	S50	S53	S57	S61	S66	S72
					12 July 1946				
0	19·45	$0·24_9$	$0·21_3$	$0·18_3$	$0·18_8$	$0·22_2$	$0·43_7$	$0·55_5$	$1·76_6$
5	15·00	$0·22_9$	$0·21_3$	$0·20_2$	$0·19_3$	$0·24_1$	$0·43_7$	$0·54_0$	$1·78_6$
10	13·93	$0·25_3$	$0·22_6$	$0·19_9$	$0·20_5$	$0·24_5$	$0·46_0$	$0·56_3$	$1·78_9$
15	13·52	$0·31_5$	$0·24_4$	$0·22_1$	$0·20_8$	$0·26_2$	$0·44_6$	$0·56_3$	$1·77_0$
20	13·41	$0·30_0$	$0·25_8$	$0·21_6$	$0·21_0$	$0·25_2$	$0·47_0$	$0·57_3$	$1·75_7$
25	12·94	$0·41_6$	$0·35_4$	$0·31_7$	$0·28_8$	$0·31_2$	$0·51_0$	$0·61_5$	$1·81_0$
					26 August 1946				
0	—	$0·92_6$	$0·83_1$	$0·79_7$	$0·75_4$	$0·80_5$	$1·00_2$	$1·13_5$	$2·31_5$
5	—	$0·83_2$	$0·75_0$	$0·72_8$	$0·70_8$	$0·71_9$	$0·86_0$	$1·03_3$	$2·15_1$
50	—	$0·82_6$	$0·72_0$	$0·68_9$	$0·70_8$	$0·70_4$	$0·87_6$	$0·99_8$	$2·15_3$
					24 October 1946				
0	14·0	$0·78_9$	$0·70_0$	$0·67_3$	$0·58_4$	$0·56_3$	$0·73_7$	$0·84_4$	1·99
5	14·1	$0·69_8$	$0·69_5$	$0·60_3$	$0·55_8$	$0·54_7$	$0·72_2$	$0·82_5$	1·95
10	14·2	$0·68_4$	$0·63_1$	$0·54_6$	$0·50_3$	$0·53_2$	$0·70_1$	$0·79_6$	1·95
15	14·2	$0·70_8$	$0·65_8$	$0·56_3$	$0·48_8$	$0·52_5$	$0·70_0$	$0·81_5$	1·97
					30 October 1946, 14.40 h				
0	—	$1·45_4$	$1·26_4$	$1·08_2$	$0·98_5$	$0·96_6$	$1·03_7$	$1·10_8$	$2·25_7$
5	—	$1·41_7$	$1·27_2$	$1·13_8$	$1·01_7$	$1·00_5$	$1·10_0$	$1·21_4$	$2·30_9$
					6 November 1946				
0	13·68	$0·17_4$	$0·15_3$	$0·14_1$	$0·12_8$	$0·14_8$	$0·34_2$	$0·47_0$	$1·61_0$
5	13·68	$0·37_4$	$0·37_3$	$0·34_6$	$0·33_0$	$0·33_0$	$0·53_4$	$0·62_7$	$1·78_1$
25	13·68	$0·24_1$	$0·23_1$	$0·18_8$	$0·18_2$	$0·22_7$	$0·41_4$	$0·52_2$	$1·69_5$
50	13·68	$0·39_6$	$0·38_3$	$0·35_4$	$0·33_3$	$0·33_9$	$0·51_8$	$0·61_5$	$1·78_3$
70	13·68	$0·38_2$	$0·36_9$	$0·35_9$	$0·29_8$	$0·31_1$	$0·51_2$	$0·61_6$	$1·76_5$

5 and 50 m. Surface samples are often more turbid than the waters beneath while phosphate analyses there are often anomalous. It is not known how thick this more turbid surface rind of water was, probably it did not exceed 1 m. Consequently the surface Pulfrich measurements cannot be compared with any submarine photometric measurements. For the upper waters only a single set of figures at 5 m is available for calculation of ratios and the excellent set of submarine measurements loses some of its value.

On the surface sample the effect upon settling of suspended matter of time of standing in the Pulfrich absorption tube was measured. The sample was collected at 15.45 h, transferred to the absorption tube and examined by Dr Atkins at 21.10 h for the extinction coefficient as measured, μ_M. Subsequent measurements were made as shown in Table 5.

TABLE 5. CHANGES IN EXTINCTION COEFFICIENTS (μ_M) ON STANDING

Water from 0 m depth, 26 August.

Treatment	Filter: Violet S43	Blue S47	Deep Red S72
Water freshly transferred to tube at 21.10 h	0·92	0·83	0·73
Following morning after standing in tube overnight undisturbed	0·71	—	0·55
After shaking up in the tube but without creating air bubbles	0·86	0·76	0·65

These results show the need for speed once the sea water has been transferred to the absorption tube. They also reveal some physical agency in the uppermost metre of water opposing the tendency of light-scattering particles to sink.

24 *October* 1946. Coastal water off Wembury in 33 m depth, 50° 17′ N., 4° 05′ W. Afternoon, photometer not now known. The water here was more turbid than in the open Channel, being most so near the surface (Table 4). Mean values have been evaluated by both methods for the stratum between the surface and 15 m depth.

30 *October* 1946. Moored to no. 3 buoy inside east end of Plymouth Breakwater. Depth 11 m. Grey cloud 10/10. Samples for Pulfrich measurements were taken first at 11.35 h and again at 14.40 h when the turbidity was found to have increased considerably. Measurements with the submarine photometer were made between 13.38 and 14.25 h and are best compared with the afternoon Pulfrich determinations given in Table 4.

6 *November* 1946. English Channel, station E1. Cubical submarine photometer. The weather was too rough for surface light-measurements. The water was isothermal to the bottom and the measurements with the cubical photometer also suggested homogeneous water. Consequently the discrepancy between the 0 and 25 m Pulfrich measurements and the other three are hard to understand. In default of a better explanation the discrepancy has been attributed to settling and the 0 and 25 m results rejected.

Comparison of extinction coefficients determined near Plymouth in 1946 *with the Pulfrich photometer and with submarine photometers*

Pulfrich measurements are made on samples from spot depths whereas the submarine photoelectric measurements are made over a range of depth.

Both are subject not only to experimental error but to the inherent variability in transparency always likely in a vertical column in the sea. The only way to compare such measurements is to take some sort of mean figure obtained by each method over a range of depth within which optical properties were shown to be reasonably uniform. For the Pulfrich instrument this means the arithmetic mean of extinction coefficients obtained on several samples from depths within the range. For submarine photoelectric measurements where, say, four measurements of light intensity at different depths were made, these were bracketed two at a time to give six values for the extinction coefficient. The mean of these six values or 'brackets' is considered to be the best measure of the extinction coefficient of the layer in question. On some occasions the available measurements fall short of requirements.

TABLE 6. COMPARISON OF EXTINCTION COEFFICIENTS IN GREEN LIGHT (530 mμ) MEASURED WITH THE PULFRICH PHOTOMETER AND BY SUBMARINE PHOTOMETER ON 12 JULY 1946

By Pulfrich photometer with spectral filter S 53		By submarine photoelectric photometer with spectral filter VG 9		Ratio,
Depth of water sample (m)	$\mu_{SW,P}$	Depth range (m)	μ_v	$\frac{\mu_{SW,P}}{\mu_v}$
0	0·188	1–5	0·065	
5	0·193	1–10	0·059	
10	0·205	1–15	0·064	
15	0·208	5–10	0·054	
—	—	5–15	0·064	
—	—	10–15	0·073	
Mean 0–15	0·199 ± 0·008	Mean 1–15	0·063 ± 0·006	3·15 ± 0·32
15	0·208	15–20	0·110	
20	0·210	15–25	0·116	
25	0·288	15–40	0·121	
—	—	20–25	0·121	
—	—	20–40	0·123	
—	—	25–40	0·124	
Mean 15–25	0·235 ± 0·037	Mean 15–40	0·119 ± 0·005	1·97 ± 0·32

To illustrate the method all the observations on 12 July 1946 are set out in Table 6 with standard deviations. All the available data are summarized in Table 7. In the last column the ratios of extinction coefficients determined by the two methods are shown with standard deviations of the ratio when assessable. Although, due to the small samples of raw data, the statistical validity of these standard deviations cannot be rated high, it is considered that they show the order of magnitude of the variability inherent in the ratios. They are unsuitable for statistical analysis such as analysis of variance.

For the different colours the arithmetic means and the ranges of the ratios are collected in Table 8.

The ratios of extinction coefficients measured with the Pulfrich photometer to the coefficients measured by a submarine photoelectric photometer ranged between 2 and 6 for green light and between 2 and 5 for blue violet. The results for 26 August which included submarine measurements with five filter combinations show the decrease in ratios from green through yellow to red and are presented in a separate column. The variation amongst these

TABLE 7. COMPARISON OF EXTINCTION COEFFICIENTS MEASURED BY THE PULFRICH PHOTOMETER AND BY SUBMARINE PHOTOMETERS

Date 1946	Depth range (m)	Maximum transmission of filters (mμ)	Extinction coefficients measured with Pulfrich photometer: Number of samples	$\mu_{SW,P}$	Vertical extinction coefficients measured with submarine photoelectric photometers: Number of brackets	μ_v	Ratio, $\frac{\mu_{SW,P}}{\mu_v}$
Blue violet ...			S43 filter		Corning blue filter		
24 Oct.	0–15	430	4	0·72 ± 0·04	6	0·23 ± 0·02	3·1 ± 0·3
30 Oct.	0–5	430	2	1·45 ± 0·05	1	0·43	3·4
Blue ...			Mean of S43 and S47 filters		BG12 filter		
12 July	0–10	450	3	0·23 ± 0·01	1	0·087	2·6
12 July	10–20	450	3	0·27 ± 0·02	1	0·11	2·4
12 July	20–40	450	2	0·33 ± 0·04	3	0·17 ± 0·00	2·0 ± 0·3
26 Aug.	1–10	450	1	0·79	3	0·15 ± 0·02	5·1
26 Aug.	40–50	450	1	0·77	1	0·17	4·7
Green ...			S53 filter		VG9 filter		
12 July	0–15	530	4	0·20 ± 0·01	6	0·063 ± 0·006	3·2 ± 0·3
12 July	15–40	530	3	0·24 ± 0·04	6	0·12 ± 0·005	2·0 ± 0·3
26 Aug.	0–15	530	1	0·71	6	0·12 ± 0·011	6·0
26 Aug.	30–50	530	1	0·71	3	0·14 ± 0·004	5·0
6 Nov.	5–70	530	3	0·32 ± 0·02	10	0·090 ± 0·004	3·6 ± 0·3
Green ...			S53 filter		Corning green filter		
24 Oct.	0–15	530	4	0·53 ± 0·04	6	0·19 ± 0·04	2·8 ± 0·6
30 Oct.	0–7	530	2	1·00 ± 0·02	5	0·25 ± 0·11	4·1 ± 1·8
Yellow ...			S57 filter		OG2 + VG9 combination		
26 Aug.	1–15	570	1	0·72	6	0·17 ± 0·01	4·3
Red ...			S61 filter		RG1 filter		
26 Aug.	1–10	620	1	0·86	3	0·40 ± 0·02	2·2
Red ...			S66 filter		Corning red filter		
24 Oct.	0–5	666	2	0·83 ± 0·01	3	0·57 ± 0·05	1·5 ± 0·1
30 Oct.	0–5	666	2	1·16 ± 0·05	1	0·61	1·9
Red ...			S66 filter		RG5 filter		
26 Aug.	1–10	666	1	1·03	3	0·60 ± 0·08	1·7

ratios much exceeds the standard deviation on any single ratio and unquestionably illustrates a fundamental property associated with the variable presence of scattering particles. With red light the much smaller absolute values of the ratio and the smaller range accord with this explanation.

TABLE 8. VARIATION WITH WAVE-LENGTH OF RATIOS OF EXTINCTION COEFFICIENTS MEASURED (*a*) WITH THE PULFRICH PHOTOMETER AND (*b*) WITH THE SUBMARINE PHOTOMETER

Colour	Wave-length (m)	26 August 1946 only	Mean of all ratios	Range of ratios
Blue-violet	430–450	5·1, 4·7	3·4	2·0–5·1
Green	530	6·0, 5·0	3·8	2·0–6·0
Yellow	570	4·3	—	4·3
Red	620–680	—	1·8	
Red	620	2·2	—	1·5–2·2
Red	680	1·7	—	

TABLE 9. MEAN RATIOS

Ratio	Green light	Red light
$\frac{\mu_{SW}\ (Pulfrich\ photometer)}{\mu_{SW}\ (Brightness\ profile)}$	1·80	1·32
$\frac{\mu_{SW}\ (Pulfrich\ photometer)}{\mu_{v}\ (Submarine\ photometer)}$	3·79	1·96
$\frac{\mu_{SW}\ (Brightness\ profile)}{\mu_{v}\ (Submarine\ photometer)}$	2·2	1·5

In Table 9 are presented mean ratios of coefficients measured with the Pulfrich photometer and the brightness profile or submarine photometric methods. From these a third ratio is derived, namely the coefficient measured by the brightness profile method against that measured by the submarine photometer. This third ratio includes a number of accumulated errors and natural variations but there is little doubt that extinction coefficients for both red and green light measured by the brightness profile method were considerably larger than the submarine photometric method of Poole & Atkins would have revealed.

DISCUSSION

For studies on the biological effects of underwater illumination in the sea the method which gives the lowest extinction coefficients and is not hampered by geometrical artifacts would seem to be the best, i.e. measurements with a photoelectric submarine photometer. In this, the flashed opal collects all the light within a wide half-angle approaching 90°.

In the brightness profile method light scattered at random will not be recorded by the aerial camera. This registers only light which leaves the sea normal, or nearly normal, to the surface. The loss will appear as an increase in the apparent extinction coefficient. As against this, some light will be

back-scattered before it reaches the sea bed. For this the length of path in water will be less than twice the depth, the effect being to decrease the apparent extinction coefficient. In coastal water of the English Channel it has been shown that the brightness profile method yields extinction coefficients greater than the submarine photometer by a factor of about 2·2 for green light and about 1·5 for red light.

In the Pulfrich photometer the light traverses the water once only so that there is no change in apparent extinction coefficient due to back scattering. The discrepancy with the submarine photometric method is even greater and is very variable, being much dependent on the quantity and nature of the scattering particles. The factor averages about 3·8 for green light and 2·0 for red light.

The conclusions from this investigation in no way invalidate Moore's brightness profile method for determining beach gradients, shown by internal evidence to be very well suited for the purpose for which he designed it.

Methods in which optical measurements are made on sea-water samples confined in tubes are still (1960) in common use. Though these methods may be internally consistent, a study of the geometry of the light paths through the instruments (cf. Appendix) is essential if results obtained with them are to be compared with others observed directly on unconfined water in the sea.

SUMMARY

Three methods of determining extinction coefficients of light in sea water have been compared: (*a*) on water samples contained in a Pulfrich photometer, (*b*) from 'brightness profiles' determined by aerial photographic survey, and (*c*) by photoelectric underwater measurements.

The ratios of extinction coefficients for green and red light as determined in 1945 by the Pulfrich photometer and by the brightness profile method are given in Table 3, columns 9 and 10. For green light the ratio is about 1·8 and for red light 1·3.

The departure from unity is attributed to the geometry within a confined tube and, within the Pulfrich measuring assembly, of light rays scattered forward by particles present in sea-water samples.

In the following year, 1946, comparisons were made between the Pulfrich method and the submarine photometric method of Atkins & Poole. The discrepancies were variable and even larger, the ratios averaging 3·8 for green light and 2·0 for red light.

The brightness profile and submarine photometric methods have not been directly compared, but, by comparing the two sets of determinations against the Pulfrich photometer, it would seem that extinction coefficients determined with the brightness profile method are about 2·2 times those determined by the submarine photometer in green light and about 1·5 times in red light.

Discrepancies in extinction coefficients of light in sea water as determined in a Pulfrich photometer with long absorption tubes compared with coefficients determined by other methods are explained in the Appendix as due to forward scattering, combined with internal and specular reflexion and the geometry of the measuring assembly of the instrument.

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APPENDIX

The effects on measurements of light extinction by natural waters of scattering and internal reflexion in long absorption tubes

Extinction coefficients for light on samples of sea water confined in metre-long tubes in a modified Pulfrich photometer have been found to be much greater than coefficients on similar water determined directly in the sea, either by photoelectric submarine photometers or by aerial photography of 'brightness profiles' by Moore (1947). An explanation, long elusive, was provided by the work of Atkins & Poole (1952) on forward scattering.

In the Pulfrich photometer the beam of light may be somewhat divergent; again, in a natural water scattering may occur. Let us however consider the fate of a parallel beam of light scattered at the point P in water contained in a clear glass tube (Fig. 1).

Since the refractive index of glass is greater than that of water, total internal reflexion will never occur at the inner glass–water interface. Specular reflexion will take place, but will be very small unless the angle with the axis of the tube is very low.

Light scattered from the point P at a high angle with the axis of the tube will pass right through the glass wall and be lost completely. As the angle with the horizontal decreases the critical angle will be passed; then all light will be totally reflected from the outer or glass–air surface back into the water through which it will take a zig-zag course due to successive internal reflexions from the outer wall. Although the total reflexion takes place at the glass–air interface, a simple exercise in geometrical optics will show that the critical angle in question is that of water against air, $\mu = 1{\cdot}34 = 1/\sin 48°$. The limiting angle with the horizontal is the complement of this angle: 42°.

Atkins & Poole (1952) have established that much of the light that is scattered in sea water is scattered forward at an angle less than about 42–45°, i.e. little light will be completely lost from the tube. Such scattered light will repeatedly be internally reflected in the absorption tube and will finally approach the end-plate within a cone

of half-angle 42°. Since the end-plate is at right angles to the axis of the tube, the half angle of the cone of light after emergence will be even greater than it had been in the tube, $\sin^{-1}\sqrt{(\mu^2-1)}$ or 63°. Little forward scattered light has been lost for a suitable integrating instrument could measure it. In consequence forward scattering seemed not to be an adequate explanation of the observed discrepancies until Dr H. H. Poole, in conversation, pointed out the crucial importance of the geometry of the measuring assembly of the Pulfrich photometer as illustrated in *Zeiss Directions for the Use of the Pulfrich Photometer* (no date), fig. 8. From this it is seen that the arrangement of diaphragms in that photometer will restrict the light received through the spectral filter by the eye to that which has emerged from the absorption tube as an essentially parallel beam. The cone of scattered, internally and specularly reflected light will be intercepted by diaphragms and by the inner walls of the photometer housing. It will be lost just as effectively as though it had been scattered through the glass walls of the absorption tube.

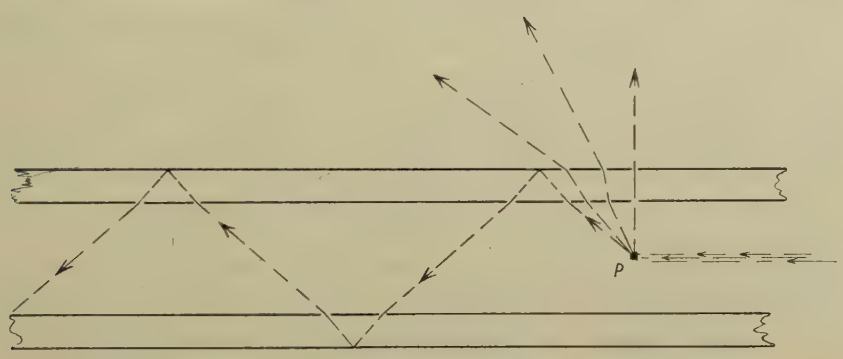

Fig. 1

When the tubes, full of air or water, are viewed lengthways against a light source, strong reflexion from the walls is seen. Whether this is due to total reflexion from the outer glass–air surface or to specular reflexion from the inner glass–water surface has not been established nor does it matter.

This is no criticism of the Pulfrich photometer for the purpose for which it was designed—the measurement of colour; but it is dangerous to use it to measure phenomena which involve scattering of light, especially in long tubes.

Although measurements of absorption plus scattering made with the Pulfrich photometer adapted for use with long tubes do not represent events which occur in the sea, they are comparable with others made with the same apparatus and have value. Other workers have combined long absorption tubes with photometric devices. For these also it would be wise to examine the geometric path of light through the whole assembly to assess the effect of forward scattering.

For water of similar scattering power in shorter tubes, 25 cm long or less, as supplied by the makers, the effect should be much less. As against this, even in short tubes, turbid solutions with much scattering would be expected to show markedly higher extinction coefficients than they would if measured unconfined.

GLOSSARY OF SYMBOLS

a, b	Reading of right-hand Pulfrich photometer drum when right-hand drum is filled with distilled water/sea water.
c_λ	Transmission of a spectral filter at a wave-length λ.
D	A transmission measured with the photometer.
g_1, g_2	Arbitrary readings of the left-hand drum of the Pulfrich photometer.
H_2O	As a suffix, stands for distilled water or pure water in contrast to sea water *SW*.
I_{H_2O}	Intensity of luminous flux in distilled water.
I_{SW}	Intensity of luminous flux in sea water.
$\mathcal{J}_\lambda$	Relative energy of a light source at a given wave-length λ.
l	Length of absorption tube.
V_λ	Relative visibility at a wave-length λ (relation between luminous sensation as perceived by the eye and radiant energy).
Y_λ	Relative sensitivity of Kodak aero Super XX film to mean noon sunlight.
Z_λ	Transmission of a Wratten filter at a wave-length λ.
λ	Wave-length of monochromatic light.
μ	(i) A micron; (ii) any extinction coefficient of light in water.
μ_{H_2O}	Any extinction coefficient for pure or distilled water.
μ_M	Extinction coefficient of sea water relative to distilled water as measured visually with a spectral filter (see equation (1)).
μ_P	Extinction coefficient determined in the Pulfrich photometer.
μ_{SW}	Absolute extinction coefficient of sea water.
μ_{S43}, etc.	An extinction coefficient determined with spectral filter S 43, etc.
μ_v	Vertical extinction coefficient in sea water determined with a submarine photometer.
μ_λ	Extinction coefficient for monochromatic light of wave-length λ.

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THE ACCUMULATION OF RADIOACTIVE CAESIUM IN CRABS

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(Text-figs. 1–7)

The amount of caesium in sea water is very small, and a value of 0·4–1·3 μg/l. has been given by Ishibashi & Hara (1959). It has been found in experiments with the fission product ^{137}Cs that Cs can penetrate into and is concentrated by marine animals (Chipman, 1960). For instance, after long exposure to ^{137}Cs, the prawn *Palaemonetes pugio* showed a concentration factor of about 25. The majority of experiments on the penetration of Cs into tissues have been carried out in vertebrates. Lubin & Schneider (1957) demonstrated that almost all of the K in isolated frog sartorius muscle can be replaced by Rb or Cs with only slight accumulation of Na. In competition experiments, with equal external concentrations, the steady-state level for tissue Cs was distinctly lower than for K. Using radioactive isotopes, Mullins (1959) has measured the relative permeability of frog sartorius muscle to alkali cations. The order of permeabilities is K > Rb > Cs > Na. Relman, Lambie, Burrows & Roy (1957) found that in young rats the muscle cells accumulate alkali metals, introduced by feeding, in order of preference Cs > Rb > K. The tissue/plasma concentration ratio for Cs which was finally reached was more than 4·5 times that for K. This fact was given as evidence against the normal uptake of K by tissues being solely the result of passive movements along an electrical gradient produced by a 'sodium pump'. Generally the evidence indicates that Cs behaves or is treated by tissues in a way which, except in degree, is similar to K.

The accumulation of radioactive Cs has been studied in six species of crabs. Much of the work has been carried out using *Carcinus maenas* (L.). In addition, three other members of the family Portunidae have been examined, viz. *Portunus puber* (L.), *P. depurator* (L.) and *Polybius henslowi* Leach. *Cancer pagurus* L. and *Corystes cassivelaunus* (Pennant) have also been used. An indication of the relative permeability of the body surfaces of different crabs to salts was given by Margaria (1931) who followed the fall in blood osmotic pressure of animals placed in dilute sea water. His results showed that both species of *Portunus* are stenohaline while *Carcinus* is euryhaline. The order of permeability of the body surface is *P. depurator* > *P. puber* > *Carcinus*. Nagel (1934) showed that the stenohaline *Portunus holsatus* is much more permeable to iodide than the more euryhaline *Carcinus* and *Cancer*. *Polybius* and *Corystes*

are probably stenohaline crabs. Accurate analyses of the inorganic constituents of the blood and urine of *Carcinus* were made by Webb (1940). He concluded that the maintenance of K in the blood at a level rather greater than that in sea water is due to active absorption of K over the body surface coupled with the excretion of urine containing less K than the blood. A similar regulation of K was found in *Cancer*, *Portunus puber* and *P. depurator* by Robertson (1939, 1953). The maintenance of the high K content of *Carcinus* muscle was studied by Shaw (1958), who found that within the limits of error K was distributed passively across the fibre membrane according to the electrochemical gradient. He suggests that there may be a very small amount of active K uptake by muscle fibres particularly at low blood concentrations.

The accumulation of trace amounts of Cs is of interest when the biological aspects of radioactive waste disposal are considered. From this point of view the isotope ^{137}Cs is most important because it has a half-life of 30 years. In a natural environment an isotope such as ^{137}Cs can enter the crab by way of the body surface or via the gut either by feeding or drinking. Most of the experiments to be described were carried out on starved animals. As drinking in intermoult crabs is not recognized as in any sense a regular process, it is assumed that most of the Cs must enter starved animals through the gills. Some experiments have been carried out in which fed animals were used and the absorption of radioactive Cs from food has been examined. The relationship between the behaviour of Cs and K ions has been studied by comparing the ways in which crabs take up the radioactive isotopes ^{134}Cs and ^{42}K. At equilibrium the levels attained by these isotopes in blood and tissues have been compared with the inactive K concentrations. A comparison of the excretion of ^{134}Cs and inactive K has also been made.

MATERIALS AND METHODS

Carcinus maenas, *Portunus puber* and *Cancer pagurus* were collected from the shore in Plymouth Sound. *Portunus depurator* and *Corystes cassivelaunus* were trawled from deeper water and *Polybius henslowi* was taken in hand-nets over deep water in the Bay of Biscay. Nearly always male animals were used.

Isotopes were obtained from the Radiochemical Centre, Amersham. ^{134}Cs was received as 0·2 g of carbonate having an activity of 1·0 mc. ^{42}K was received as 1·0 g of carbonate having an initial activity of about 2 mc. The carbonates were converted to chlorides with dilute hydrochloric acid and after evaporation 100 ml. stock solutions were made up with distilled water.

For isotope experiments partitioned glass tanks of about 5 and 10 l. capacity were used. Each was covered with a close-fitting lid through which passed one tube for aeration and a second tube carrying exhaust air outside the laboratory. About 2 l. of experimental medium were allowed per animal, and in long experiments this was changed at intervals. The media used were normal

artificial sea water and artificial sea water with half the normal K content. Artificial sea water (Cl‰ = 19) was prepared according to the tables of Barnes (1954). In ^{134}Cs-uptake experiments a sea water activity of 5 μc/l. was used and with ^{42}K the initial activity was about 100 μc/l. These isotope concentrations gave the sea water a Cs level of about 0·003 mM/l. or an additional K concentration of 0·3–0·4 mM/l. The work was done at a room temperature of 16–18° C or in a regulated tank at 17° C.

Prior to uptake experiments animals were allowed 2 days to become equilibrated with inactive artificial sea water. To measure the activity of a whole animal it was drained of medium, placed in a 200 ml. beaker and counted within a ring of 8 γ-sensitive GM tubes (20th-Century type G. 10. Pb). This equipment was used by Fretter (1955). At intervals samples of medium were counted in the same beaker, and any depletion of activity was corrected by changing the solution.

Urine was taken from the excretory organ opening with a glass pipette after the operculum had been raised with a blunt needle. Blood was taken with a glass pipette from the region between the carapace and a rear walking leg. Samples of whole blood and of centrifuged blood were both used and compared. For radioactive estimations blood or urine was weighed on an aluminium planchet and the dried sample counted beneath the end window of a γ-scintillation counter (type 1186A). Other samples were diluted for K estimation by flame photometry.

Tissues for radioactive and K analysis were dissected out and dried on filter paper. Samples were not washed because Cs or K might have been selectively removed. Normally about 0·3 g of tissue was needed. Muscle was taken from the coxopodite segments of the walking legs and chelae. The water content of each tissue sample was determined in a 25 ml. conical 'Pyrex' flask. Up to 5 ml. of concentrated nitric acid were added to the dry sample in 1 ml. lots and slowly evaporated off over an electric fire element. Hot distilled water was used to dissolve the residue, occasionally with the help of a drop of acid. Blank samples were also prepared. Tissue radioactivity was measured on a 10 ml. sample placed below the end window of the γ-scintillation counter. The rest of the sample was further diluted for K estimation. In order to save time during experiments with ^{42}K the tissue water content was not determined.

In graphs for isotope uptake the activity of the whole animal, blood, urine and tissues is expressed as a concentration factor and is plotted against time in hours. The concentration factor is the ^{134}Cs activity, ^{42}K activity or the inactive K concentration per kg of tissue divided by the corresponding concentration per kg of sea water. Where uptake of isotope was followed by loss in inactive sea water, the results are still expressed as concentration factors relative to the activity of the sea water during uptake.

RESULTS

The effect of caesium on Carcinus

Preliminary experiments indicated that *Carcinus* would die within 5 days if more than 20% of the K in artificial sea water was replaced by Cs. When 20% Cs was used animals lived for up to 3 weeks and for a longer period in 10% Cs. As *Carcinus* is able to live fairly indefinitely in artificial sea water with 50% K but no Cs it would appear that Cs must be toxic at relatively high external concentrations. However, there is no reason to suspect that the 0·003 mM/l. added with ^{134}Cs would have any effect on the animal. In experiments with *Galathea squamifera* and *Palaemon serratus*, which are unpublished, it has been shown that there is no significant difference between the rates at which ^{134}Cs and the carrier free isotope ^{137}Cs are accumulated.

Some vertebrate tissues are able to function in the presence of large amounts of Cs. Relman and his co-workers (1957) found that in rats, where the normal level of K in muscle was 170 mM/l. H_2O, concentrations of Cs as high as 110 mM/l. H_2O and K concentrations as low as 50 mM/l. H_2O could be achieved before the animals died.

Uptake of ^{134}Cs *and* ^{42}K *by whole animals*

Curves showing the increase in the ^{134}Cs concentration factor with time are given for all six species in Fig. 1 A–C. Uptake of ^{134}Cs by whole animals is a slow process particularly in *Carcinus*. Analysis shows that most of the curves are roughly exponential in shape except over the first 50 h when uptake is more rapid. Comparable curves for the uptake of ^{42}K are given in Fig. 2 A–C. These curves are almost totally exponential, particularly for *P. puber* and *P. depurator*. In Figs. 1 and 2 single curves are given for each species except when fairly wide variations were found such as in *Cancer* (Fig. 1 B). The results for all animals which approached equilibrium closely are given in Table 1. As the rates of attainment of equilibrium in sea water containing ^{134}Cs are not constant they have been compared with those found with ^{42}K in terms of the time taken to reach 50% of the concentration factor when equilibrium was substantially reached. The equilibrium concentration factors and weights of the animals are also given.

From the figures and table it is immediately obvious that ^{42}K is accumulated far more quickly than ^{134}Cs in all species and that ^{42}K equilibrium concentration factors are lower than those for ^{134}Cs. Uptake of both isotopes is slowest in *Carcinus* where at equilibrium the concentration factors for ^{134}Cs are approximately double those for ^{42}K. In the other species this difference is less marked. In artificial sea water containing 50% K the whole *Carcinus* concentration factor for ^{42}K is increased and a corresponding increase in the factor for ^{134}Cs is found. The times taken for both isotopes to reach virtual equilibrium in this solution are about the same as in normal artificial sea

TABLE 1. TIMES TAKEN TO ATTAIN 50% OF THE EQUILIBRIUM CONCENTRATION FACTORS FOR ^{134}Cs AND ^{42}K IN WHOLE CRABS

(The figures are given as mean values with standard deviations.)

Species	^{134}Cs				^{42}K			
	No. of animals	Mean weight (g)	Equilibrium concentration factor	Time for 50% uptake (h)	No. of animals	Mean weight (g)	Equilibrium concentration factor	Time for 50% uptake (h)
Carcinus maenas	4	34	8·5 ± 0·6	230 ± 45	6	42	4·0 ± 0·3	19·0 ± 6·0
C. maenas in 50% K artificial sea water	3	22	13·1 ± 0·8	237 ± 49	2	27	8·0 ± 0·2	17·5 ± 2·5
Portunus puber	6	25	6·6 ± 1·4	180 ± 81	4	44	5·9 ± 0·7	3·1 ± 0·7
P. depurator	2	16	6·5 ± 0·0	110 ± 10	2	20	5·6 ± 0·1	2·5 ± 0·5
*Polybius henslowi**	1	11	5·2	50	2	9	4·2 ± 0·2	3·2 ± 0·2
Cancer pagurus	6	15	6·0 ± 1·4	89 ± 54	2	28	3·9 ± 0·2	15·5 ± 0·5
Corystes cassivelaunus	1	8	4·4	190	2	10	3·6 ± 0·1	4·2 ± 1·2

* These animals died after equilibrium appeared to have been reached.

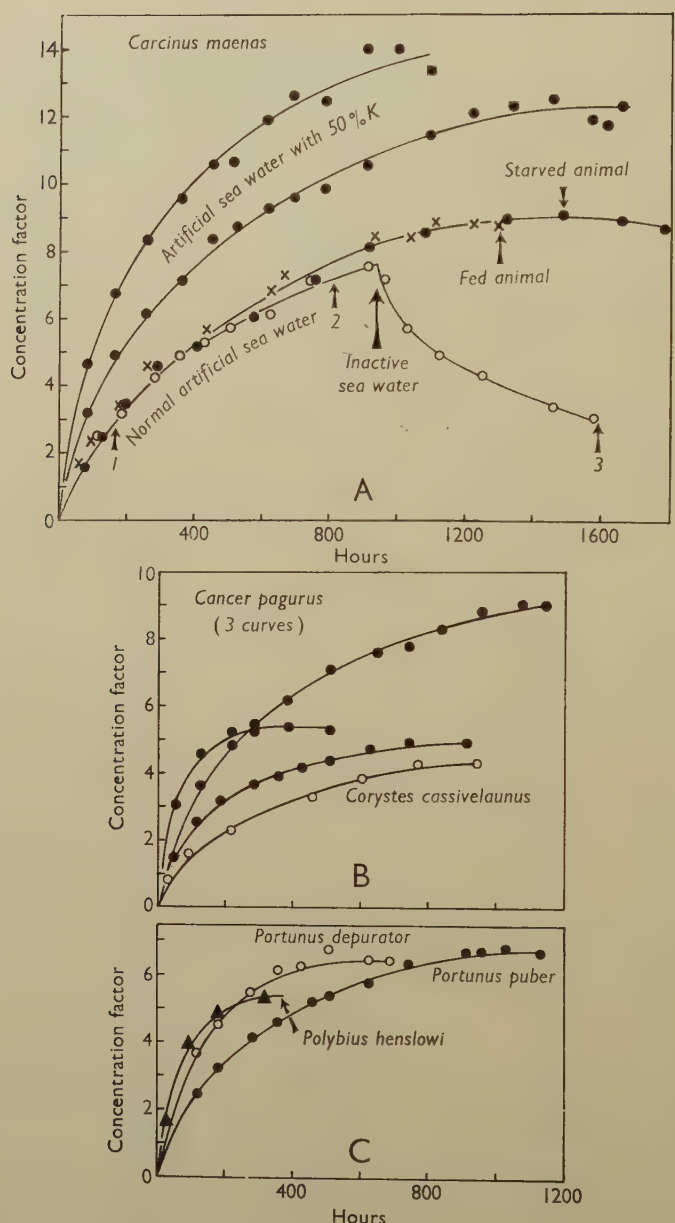

Fig. 1 A–C. Uptake of ^{134}Cs by whole crabs from artificial sea water. The concentration factor is the activity per kg whole crab divided by the activity per kg of sea water. Also, Fig. 1A, loss of ^{134}Cs by *Carcinus* in inactive artificial sea water. Arrows in the loss experiment show the concentration factors in animals for which tissue concentration factors are given in Table 6.

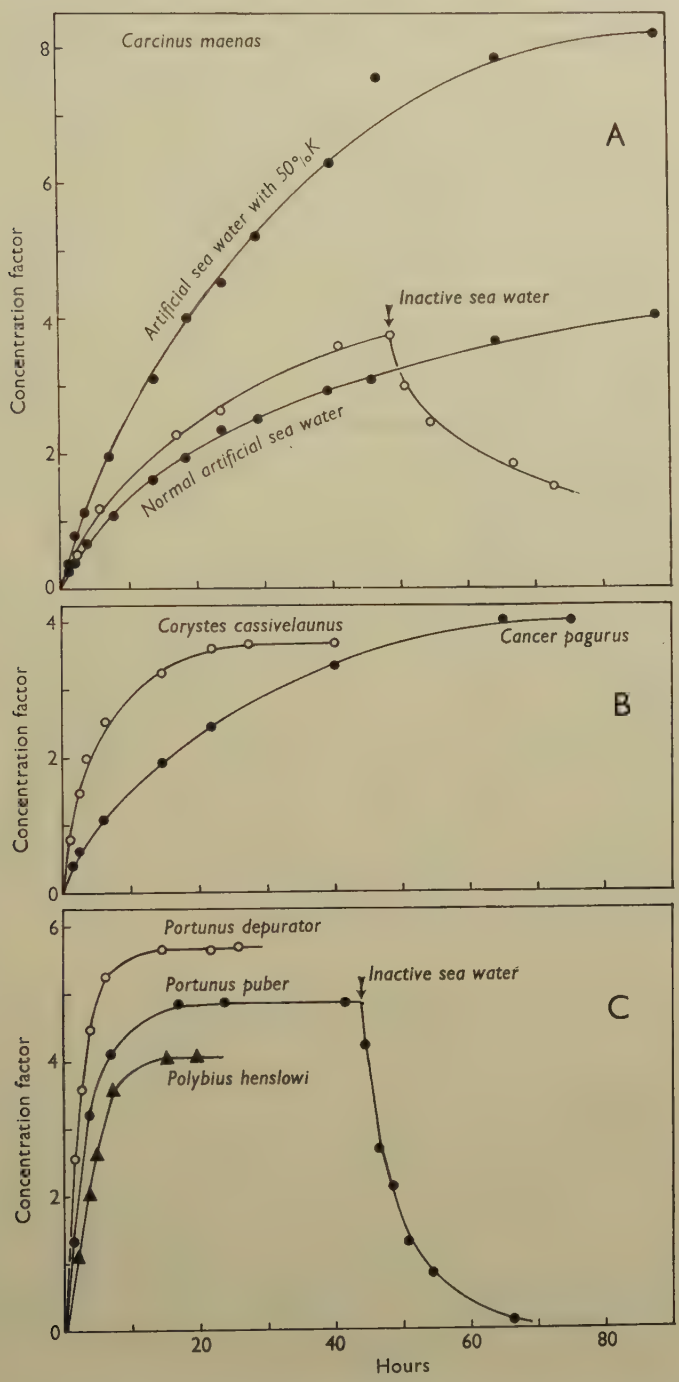

Fig. 2 A–C. Uptake of ^{42}K by whole crabs from artificial sea water. Also, Fig. 2 A, loss of ^{42}K by *Carcinus* in inactive artificial sea water. Also, Fig. 2 C, loss of ^{42}K by *Portunus puber* in inactive artificial sea water.

water. *Portunus puber* reaches equilibrium with ^{134}Cs rather more quickly than *Carcinus* but uptake of ^{42}K is much more rapid and is comparable with that found in *P. depurator* and *Polybius*. These latter species take up ^{134}Cs more rapidly than does *P. puber*. *Cancer* accumulates ^{42}K at a relatively slow rate which resembles that in *Carcinus*, while figures for ^{134}Cs vary to an extent which almost covers the whole range of values found in the other species. This variation does not appear to depend on differences in the size of specimens used. Although the experiments carried out have been comparatively few in number, results for a single species do not, except in the case of *Cancer*, show very wide variations. There appears to be no constant relationship between the rates at which the two isotopes are taken up in the different species. It would not therefore be feasible to carry out rapid ^{42}K experiments with other species and expect to be able to forecast accurately the rate of uptake of ^{134}Cs from the results. For instance 50% of the equilibrium ^{42}K level in *Carcinus* is reached about 12 times more rapidly than that for ^{134}Cs, but the corresponding value in *P. puber* is about 60 times. While in *Cancer* the average value is about 6 times, that for *P. depurator* is more than 40 times.

Animals which moulted were encountered in a few experiments. Uptake of the isotope was rather greater than normal prior to moulting. At the moult both isotopes were taken up very rapidly and equilibrium was reached in a relatively short time. For example, a specimen of *Carcinus* which moulted at the beginning of a ^{42}K experiment had reached equilibrium inside 42 h, while normally equilibrium is reached in about 100 h. Robertson (1960) has shown that in intermoult *Carcinus* ion movements probably take place across the gills as drinking is an intermittent process. At the moult sea water to the extent of 66% of the pre-moult weight is taken up through the foregut and hepatopancreas. Undoubtedly this uptake of sea water at the moult is important in the rapid uptake of isotope. It is also possible that rates of exchange between blood and tissues may have been increased.

Uptake of ^{134}Cs *by blood and tissues*

To show which part of the process of ^{134}Cs accumulation is the limiting factor in the attainment of equilibrium the blood and some of the tissues of *Carcinus* and *Cancer* were analysed at intervals over the period of uptake. Results for *Carcinus* are shown in Fig. 3 A–C and for *Cancer* in Fig. 4 A and B.

The appearance of ^{134}Cs in the blood of *Carcinus* is relatively rapid during the early stages of uptake but equilibrium is reached only slowly due presumably to ^{134}Cs being removed by the tissues. Of the organs of the body the gills and excretory organs (end sac and labyrinth) take up ^{134}Cs most rapidly. The limiting factors in ^{134}Cs accumulation by whole animals appear to be the slower rates of uptake found in the hepatopancreas, and in particular in muscle and in the vas deferens of the reproductive system. The situation in *Cancer* is not as clear, but in this crab and in the other species uptake by

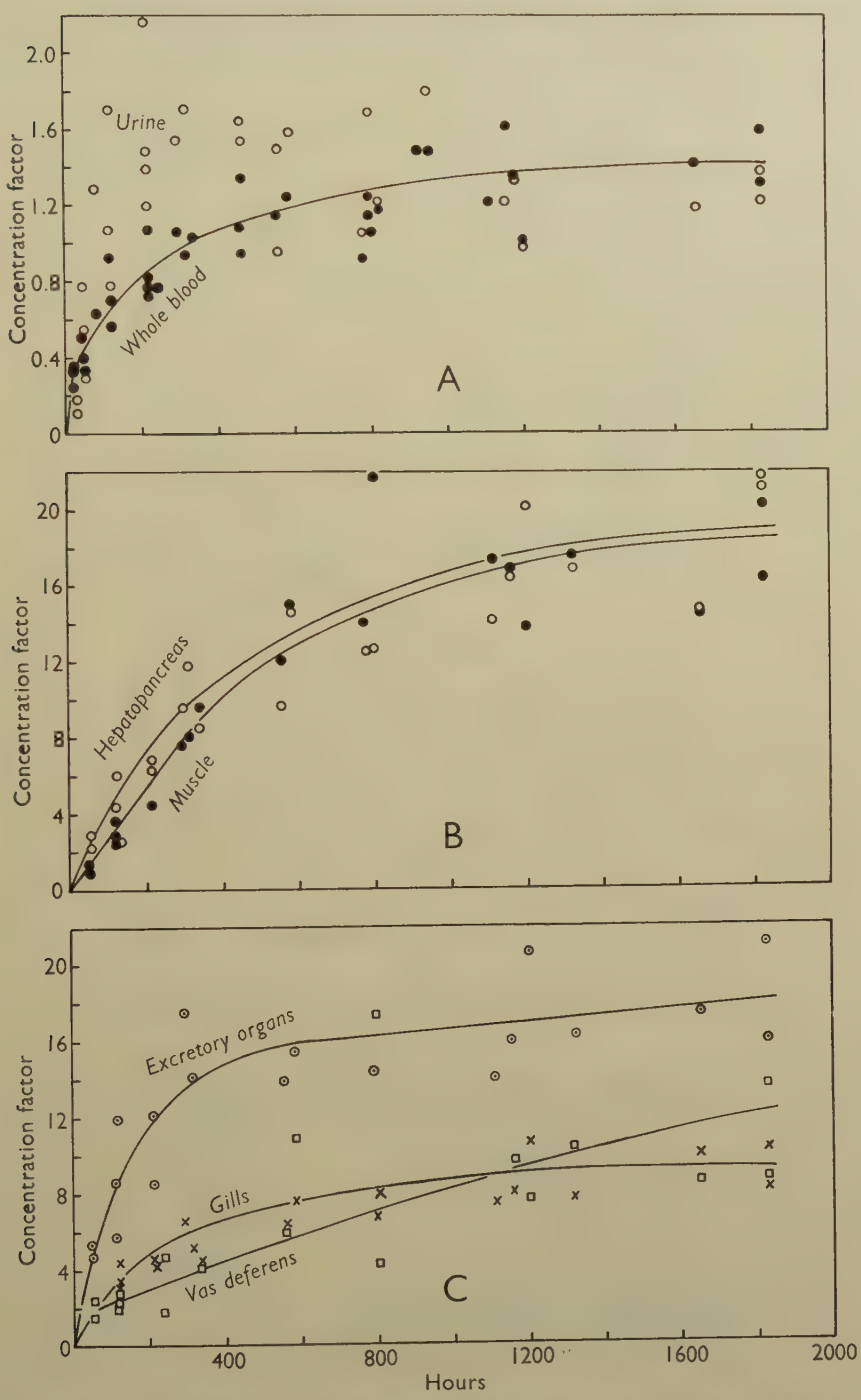

Fig. 3 A–C. Uptake of ^{134}Cs by whole blood, tissues and urine of *Carcinus maenas* in artificial sea water.

muscle is probably the process on which the attainment of whole animal equilibrium mainly depends. Table 2 shows that in *Carcinus* muscle is by far the most abundant of the soft tissues.

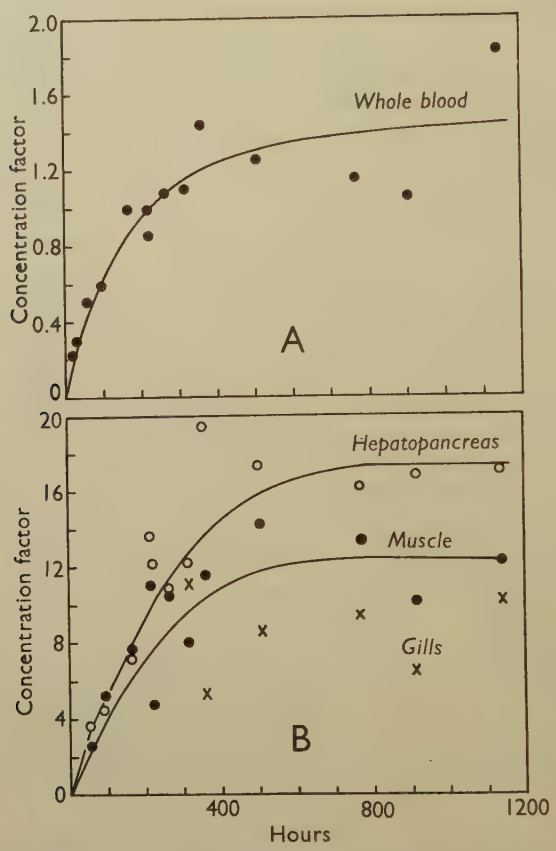

Fig. 4 A, B. Uptake of ^{134}Cs by whole blood and tissues of *Cancer pagurus* in artificial sea water.

TABLE 2. PERCENTAGES BY WEIGHT OF TISSUES IN A 32 G ♂ SPECIMEN OF *CARCINUS MAENAS*

Tissue	Percentage
Skeleton (including epidermis)	38·36
Muscle (including heart)	21·26
Hepatopancreas	6·97
Gut (including contents)	3·99
Gills	1·59
Reproductive system	0·31
Excretory organs	0·11
Fluid (mainly blood and urine)*	27·41

* Amount of fluid calculated by difference.

TABLE 3. EQUILIBRIUM CONCENTRATION FACTORS FOR ^{134}Cs AND INACTIVE K IN CRAB TISSUES

Species	No. of animals	Mean weight (g)	K (mM/kg)	% H_2O	Concentration factor K	Concentration factor ^{134}Cs	K (mM/kg)	% H_2O	Concentration factor K	Concentration factor ^{134}Cs	K (mM/kg)	% H_2O	Concentration factor K	Concentration factor ^{134}Cs
			Whole blood				Muscle				Hepatopancreas			
Carcinus maenas	5	36·1	12·7	92·5	1·20	1·43	103	78·2	9·8	17·7	100	71·5	9·5	18·7
C. maenas (sea water with 50% K)	4	20·0	7·1	—	1·31	1·60	95	80·1	17·6	42·0	90	79·0	16·6	29·6
Portunus puber	4	25·5	15·5	—	1·44	1·45	102	80·8	9·5	13·1	82	78·3	7·6	11·9
P. depurator	1	15·9	13·2	—	1·34	1·56	108	78·5	11·0	12·0	82	77·0	8·3	25·9
Cancer pagurus	5	15·2	12·3	—	1·15	1·33	84	75·2	7·9	11·3	113	78·0	10·6	19·1
Corystes cassivelaunus	1	7·7	13·8	—	1·30	1·26	112	76·7	10·6	11·4	121	75·0	11·4	24·8
			Gills				Vas deferens				Excretory organs			
Carcinus maenas	—	—	45	84·9	4·3	8·2	110	73·7	10·5	10·5	75	82·5	7·2	17·5
C. maenas (sea water with 50% K)	—	—	38	87·1	7·0	14·4	108	65·7	20·0	17·5	62	86·9	11·5	22·3
Portunus puber	—	—	46	87·6	4·3	8·9	—	—	—	—	—	—	—	—
P. depurator	—	—	31	86·9	3·1	6·0	—	—	—	—	—	—	—	—
Cancer pagurus	—	—	42	87·2	3·9	8·4	—	—	—	—	—	—	—	—
Corystes cassivelaunus	—	—	52	82·2	4·9	10·3	—	—	—	—	—	—	—	—

In animals where ^{134}Cs equilibrium appeared to have been reached the K concentrations of blood, plasma, urine and tissues were measured. The mean ^{134}Cs and K concentration factors for the tissues of five species are given in Table 3 together with mean values for tissue water content and K concentration. In nearly all cases concentration factors for ^{134}Cs exceed those for inactive K by a far greater margin in the tissues than in the blood. The result is that tissue/blood ratios for ^{134}Cs are nearly always greater than those for K. Exceptions are the vas deferens of *Carcinus*, where equilibrium was probably not reached, and in *P. depurator* muscle where the discrepancy is small. In considering the relationship between tissue and blood concentrations it is more accurate to use the relationship tissue/plasma with concentrations calculated as per kg H_2O. Samples of blood plasma had average ^{134}Cs and K concentrations about 5 % lower than those of an equal weight of whole blood. Results calculated on this basis for two specimens of *Carcinus* after 1,824 h, when absolute equilibrium appeared to have been reached, are given in Table 4. The implications of the higher plasma/sea water and tissue/plasma ^{134}Cs ratios will be considered in the discussion.

TABLE 4. TISSUE/PLASMA RATIOS FOR ^{134}Cs AND INACTIVE K IN TWO *CARCINUS* AFTER 1,824 H

(Ratios calculated using concentrations per kg H_2O.)

Tissue	1 (22·6 g)		2 (42·9 g)	
	K	^{134}Cs	K	^{134}Cs
Muscle	9·8	18·7	8·3	12·1
Hepatopancreas	13·5	22·1	9·4	22·4
Gills	4·5	6·9	4·6	7·2
Vas deferens	11·2	13·1	12·8	7·4
Excretory organs	8·1	20·7	6·4	14·9
Ratio, plasma/sea water	1·07	1·31	1·53	1·67

Loss of ^{134}Cs *by* Carcinus

After accumulating ^{134}Cs for 938 h a specimen of *Carcinus* was allowed to lose activity in inactive sea water for a further 644 h. The tissues were then analysed for ^{134}Cs and the results compared with those for an animal which had a concentration factor corresponding to the point at which loss of the isotope was commenced. A further comparison was made with an animal which had the same concentration factor but was accumulating ^{134}Cs. The full course of uptake and loss is given in Fig. 1 A and Table 5 compares the tissue concentration factors in the three animals.

The low activities of blood, excretory organs and hepatopancreas after exposure to inactive sea water (animal 3) compared with those in animal 2 show that these tissues had lost a much larger percentage of the original ^{134}Cs than had the muscle and vas deferens. Results for animal 1 show that these latter tissues also accumulated ^{134}Cs most slowly. This confirms the conclusion,

arrived at in a previous section, that muscle is the principal limiting factor in the attainment of equilibrium and also in the loss of activity. The gills were not considered in Table 5 because analyses in active sea water were carried out without washing the filaments.

Although ^{134}Cs uptake by whole *Carcinus* is roughly exponential a semi-logarithmic plot showed that the loss of isotope is not exponential because the rate of loss is gradually decreasing. This could to some extent be a result of long starvation, but it is probably due mainly to the reabsorption by the tissues of ^{134}Cs already lost to the blood.

TABLE 5. TISSUE CONCENTRATION FACTORS FOR ^{134}Cs IN *CARCINUS* AT POSITIONS INDICATED BY ARROWS IN FIG. 1A

	Animal		
	1	2	3
Whole animal	2·9	7·2	3·0
Whole blood	0·93	1·22	0·2
Muscle	3·6	17·3	7·9
Hepatopancreas	6·0	14·1	3·5
Vas deferens	2·8	9·6*	5·0
Excretory organs	11·8	13·9	3·5

* Taken from an animal of concentration factor = 8·4.

Uptake of ^{42}K *by tissues*

The tissues of crabs, for which whole animal uptake curves have been given, were analysed when ^{42}K equilibrium was reached or approached. Owing to the decay of ^{42}K the maximum length of an experiment was about 90 h. The standard deviation of ^{42}K counting was 1–1·5 % for the blood and tissues of *Portunus*. This was increased to about 5·5 % for blood and 3 % for tissues in *Carcinus* after 65 h. After 90 h the figures were about 10 % for blood and 6 % for tissues. Because of this, results for animals which were analysed after about 50 h are fairly inaccurate. Table 6 shows the inactive K concentrations of blood and tissues and the levels reached by ^{42}K are expressed as a percentage of the inactive K concentration factor. In both species of *Portunus* most of the K in the blood, muscle and hepatopancreas is readily exchangeable with ^{42}K. Similarly, by 90 h fairly complete exchange has taken place in *Carcinus* in normal and in 50 % K artificial sea water. Equilibrium appears to have been fully reached in *Cancer* after 75 h but *Corystes* was probably analysed too early at 40 h, although whole animal equilibrium appeared to have been reached.

Only in *Carcinus* has uptake of ^{42}K by the individual tissues been studied in any detail. Equilibrium with ^{42}K is substantially reached in about 10 h by the gills (Fig. 5B). In the blood, hepatopancreas and muscle equilibrium is reached only after about 90 h (Figs. 5A and 5B). It is not clear from the graphs whether uptake by the blood or by the tissues is the limiting process in ^{42}K

TABLE 6. THE PERCENTAGE EXCHANGE OF ^{42}K FOR INACTIVE K IN CRAB TISSUES AT EQUILIBRIUM

(The tissue concentration factors attained by ^{42}K are expressed as a percentage of the concentration factor for inactive K.)

				Whole blood		Muscle		Hepatopancreas		Gills	
Species	Time (h)	Weight (g)	Sea water K (mM/kg)	K (mM/kg)	% exchange of ^{42}K	K (mM/kg)	% exchange of ^{42}K	K (mM/kg)	% exchange of ^{42}K	K (mM/kg)	% exchange of ^{42}K
Carcinus maenas	65·3	32·6	11·4	10·8	107	114	89	77	96	50	98
	89·5	46·5	11·4	12·9	95	101	94	65	100	47	105
C. maenas	67·0	23·8	6·4	9·0	76	113	85	84	86	37	98
	90·0	29·5	6·4	8·4	87	105	99	96	94	42	102
Portunus puber	26·1	25·5	11·2	15·2	103	130	99	93	98	—	—
	26·5	50·1	11·2	16·4	98	131	104	96	86	—	—
P. depurator	25·5	20·1	11·2	14·7	104	107	106	120	95	—	—
	26·8	20·5	11·2	14·4	95	132	100	105	105	—	—
Cancer pagurus	75·0	26·4	11·2	12·7	72	112	123	102	117	—	—
	75·5	29·0	11·2	13·3	88	123	112	103	112	—	—
Corystes cassi-velaunus	40·0	6·8	11·2	12·3	98	121	93	84	88	—	—
	40·5	13·5	11·2	11·9	92	123	86	126	85	—	—

uptake by the whole animal. Tissue exchange is relatively rapid because by 40 h tissue/plasma ^{42}K ratios have risen to values similar to those found at equilibrium although the blood has not actually attained equilibrium with the outside medium. These ratios (calculated using concentrations per kg wet weight as the water content was not measured) are about 9–10 for muscle and

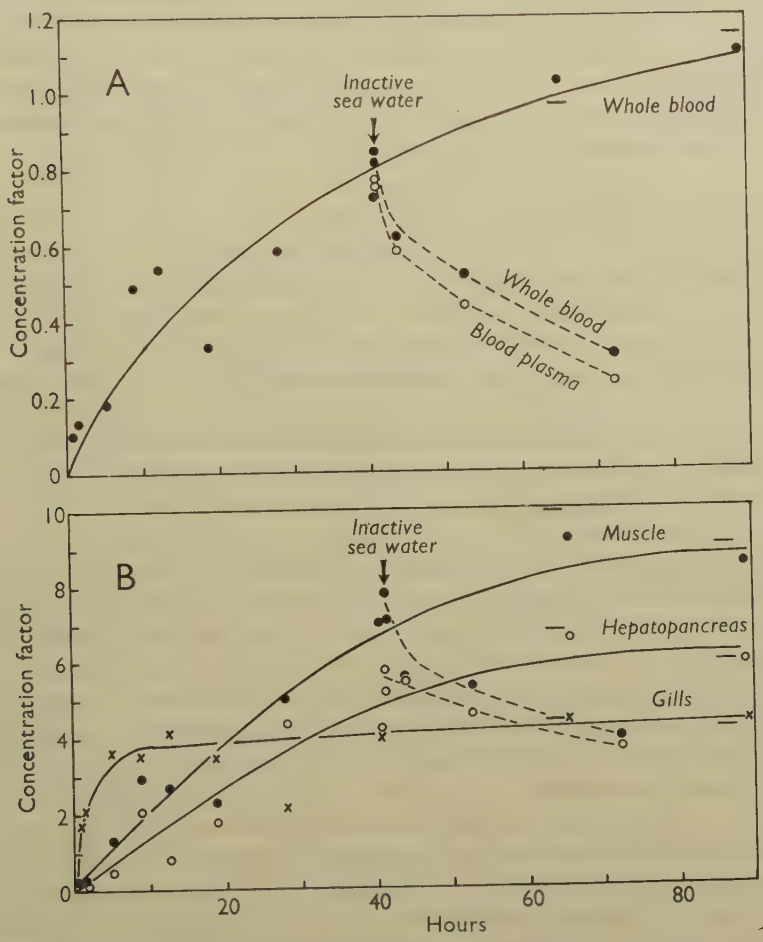

Fig. 5 A, B. Uptake and loss of ^{42}K by whole blood, blood plasma and tissues of *Carcinus maenas* in artificial sea water. Short horizontal lines indicate the inactive K levels which were found.

between 6 and 7·5 for hepatopancreas. The fact that high tissue/plasma ratios are found well before equilibrium is reached does suggest that uptake over the body surface into the blood may in fact be the limiting process. Shaw (1958) has shown that in *Carcinus* injected with ^{42}K and kept out of water,

complete exchange with muscle K takes about 6 h. When animals were immersed in sea water containing ^{42}K he found that 48 h were sufficient for equilibrium to be reached between sea water and blood and between blood and muscle. In the present experiments at least 90 h were needed for whole animal equilibration. This is presumably a physiological difference between *Carcinus* from Plymouth and Cullercoats. It would be necessary to repeat his experiments with Plymouth animals to determine whether the difference was in the rate of exchange between blood and tissues or between the blood and sea water. To try and determine what is the limiting factor in ^{42}K exchange by whole animals the loss of ^{42}K into inactive sea water has been followed and the results are given in the next section.

Loss of ^{42}K *by* Carcinus *and* Portunus

One curve for the loss of ^{42}K into inactive sea water from *Carcinus* is given in Fig. 2A. A semi-logarithmic plot of the results from this and two other experiments showed that loss is not an exponential process. Loss is most rapid initially and gradually becomes slower. This was also found in *Portunus puber* and *P. depurator* where a semi-logarithmic plot showed that the rate of loss during the latter part of each experiment was too slow for the loss curve to be described as exponential. Two experiments were carried out with each species of *Portunus* and the complete uptake and loss of ^{42}K in one specimen of *P. puber* is given in Fig. 2C.

In order to follow the loss of ^{42}K from the blood and tissues of *Carcinus* five animals were first of all allowed to absorb ^{42}K for about 40 h. When it was ascertained that they had reached similar concentration factors the activity of the blood plasma, whole blood, muscle and hepatopancreas was measured in two animals and the others were allowed to lose ^{42}K in inactive sea water. At intervals the level of ^{42}K in the blood and tissues of these animals was measured. The results of this experiment are shown in Fig. 5 with a broken line joining the points. It is found that ^{42}K is lost from the blood, presumably over the body surface, sufficiently rapidly to cause the tissue/plasma ratio for muscle to be increased from 9·6 at 41 h to 16·9 at 72 h. The increase in the ratio for hepatopancreas is from 7·0 to 16·4. Contrary to the suggestion made in the previous section this result would tend to indicate that exchange between blood and tissues is the principal limiting factor in ^{42}K exchange between the whole crab and sea water. It is therefore likely that the time constants governing the uptake of ^{42}K by the blood from sea water and by the hepatopancreas and muscle from the blood are sufficiently alike for all three to be limiting factors in the attainment of equilibrium by the whole animal.

Feeding and ^{134}Cs *uptake in* Carcinus

Feeding might have the metabolic effect of causing an increase in the rates of processes responsible for ^{134}Cs accumulation. Stobbart (1959) has shown that in the larvae of the mosquito *Aëdes aegypti* ^{22}Na is absorbed far more rapidly in fed than starved animals. In radioactive sea water feeding on inactive food might enhance uptake as a result of the absorption of isotope from fluid taken in with the food. Feeding on active food would definitely be expected to increase the rate and extent of isotope accumulation.

An example of ^{134}Cs uptake by *Carcinus* fed on inactive food in active artificial sea water is given in Fig. 1 A where it is compared with two starved animals. This 36 g crab was given as much food as it could eat before the experiment and given a total of 12 g of *Nereis diversicolor* at eight intervals over the first 1000 h of ^{134}Cs uptake. Two of these experiments were carried out and uptake curves for these and for starved animals were so similar that up to 1000 h starvation appears to have no effect. It can be seen in Fig. 1 A that the concentration factor for the starved animal falls slightly at equilibrium. This is probably due to the decreasing size of the hepatopancreas and would not be expected to be found in fed animals.

The accumulation of ^{134}Cs from food and its subsequent loss in inactive sea water has also been studied. Crabs weighing between 5 and 10 g, which had been starved for from 3 days to 2 weeks, were fed in inactive sea water on an excess of radioactive *Nereis diversicolor*. The *Nereis* had a mean concentration factor of 5·8 in sea water containing 5 μc/l. of ^{134}Cs. This is approximately the concentration factor found at equilibrium. The activity of a fed crab was measured with the animal held 5 cm below the end window of a γ-scintillation counter. Crabs consumed most of the food in the first hour and after 2 h feeding was virtually completed. The amount of *Nereis* eaten by each animal was equal, in most cases, to between 8 and 9 % of the body weight of the starved crab. This figure probably represents the maximum capacity of the crab foregut. The crabs were allowed to lose ^{134}Cs in inactive sea water and then at intervals the activities of blood, muscle, hepatopancreas and the whole of the gut were measured on wet ashed samples from different individuals. Tissue activities were corrected for the fact that the initial activity per gram of whole animal was not exactly the same in each crab.

Results in Fig. 6 A correspond to those for an animal of 9·4 g which consumed 0·68 g of *Nereis* with an activity of 29 μc/g. In actual fact Fig. 6 B shows that the animals which were analysed had lost ^{134}Cs at rather different rates but this was not taken into account. Rapid digestion and absorption of the food over the first 24 h causes the activity of blood, hepatopancreas and muscle to rise while that of the foregut falls as it empties. The fall in the activity of the whole animal is probably due to losses over the body surface and by excretion rather than to losses in the faeces. Most of the faeces were

produced over the first 48 h after feeding and their activity was low although leaching out had probably taken place. As was found in previous experiments on the loss of ^{134}Cs, the isotope was lost rapidly from the blood and more rapidly from the hepatopancreas than from muscle. By giving some animals a second radioactive meal after 100 h a marked stepwise increase in the activity of the tissues was produced. By feeding in a contaminated environment *Carcinus* would be expected to shorten the time taken to reach equilibrium with the sea water and also to enhance the level eventually reached.

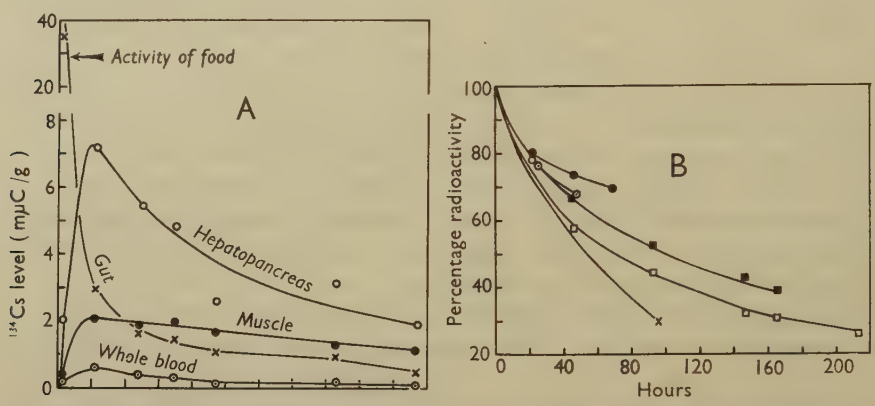

Fig. 6 A. Changes in the ^{134}Cs activity of *Carcinus* blood and tissues following a single meal of radioactive *Nereis diversicolor* in inactive sea water. The results are for an animal of 9·4 g which ate 0·68 g of *Nereis*. Lower scale represents hours, as in B.

Fig. 6 B. Loss of ^{134}Cs in inactive sea water by six *Carcinus* the tissues of which were used for the analyses in Fig. 6 A.

Excretion of ^{134}Cs *and* K

In *Carcinus* the level of ^{134}Cs in the urine has been measured in animals exposed to the isotope for different lengths of time. These results, for one excretory organ only, are shown as concentration factors in Fig. 3 A. In many cases the levels of ^{134}Cs in urine after 200 h are as high as or higher than values found when the blood activity had further increased and equilibrium had been reached. To check that this was not a result of individual variation, simultaneous plasma and urine samples were taken from the same individuals over a period of up to 1200 h. Blood samples of about 0·02 ml. were taken from the region between the carapace and rear walking leg with a fine syringe with the result that very little blood escaped from the animal. The blood was centrifuged and the plasma sample weighed and dried on a planchet before counting. Fig. 7 shows the corresponding blood and urine ^{134}Cs concentrations relative to sea water calculated as per kg H_2O which were found during uptake in three animals.

In each case the urine activity at about 200 h is two or more times that of the blood while after 800 h it has fallen to a level only slightly greater than that of the blood. It was thought that this rather odd result might be due to the long period of starvation endured by these animals. One of the animals was fed three times on as much inactive *Nereis diversicolor* as it could consume but the rise in activity of the urine which resulted was very small. Two more shorter experiments were carried out in which the animals were fed. In both cases the activity of the urine fell relative to that of the blood during uptake.

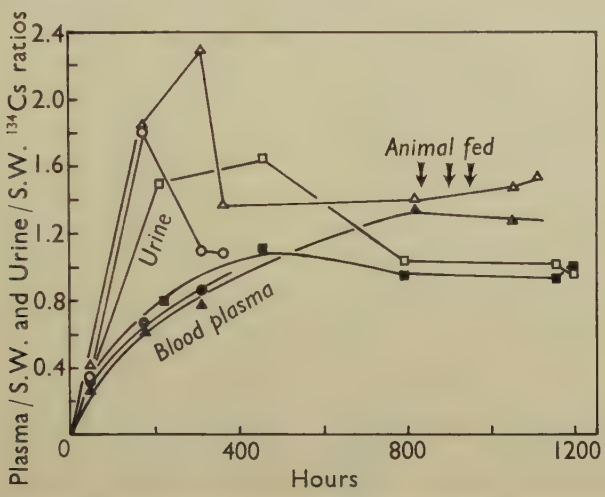

Fig. 7. Corresponding plasma/sea water and urine/sea water ^{134}Cs ratios (from concentrations calculated as per kg H_2O) measured during the uptake of ^{134}Cs by three *Carcinus* from artificial sea water.

No connexion could be found between these changes in urine activity and in the total activity of the excretory organ which increased gradually up to equilibrium (Fig. 3 c).

Results for *Carcinus* in which both ^{134}Cs and K levels of plasma and urine were measured are shown in Table 7. These figures show how the urine/plasma ratio changes during uptake. In the initial stages of uptake the urine activity may be less than that of the blood due to its being diluted by inactive urine present at the start of uptake. The urine activity then rises until it is about twice that of the blood. Later the urine activity falls to a level similar or sometimes less than that of the blood. During these changes urine/plasma ratios for K remain constant at about 1·0. After 1,200 h the plasma/sea water ratios for ^{134}Cs exceed those for K and both values are greater than 1·0. In animals which had reached equilibrium in artificial sea water with 50% K the results are generally similar but the plasma/sea water ratios for both ions are on average rather higher.

Measurements which were made in *P. puber* and *Cancer* at equilibrium are

also shown in Table 7. In *Portunus* the mean urine/plasma ratio for K was 0·77 and a value of 0·89 was found in *Cancer*. The corresponding values for ^{134}Cs in both animals were markedly greater and in four out of five cases exceeded 1·0. Where urine samples were taken during the earlier stages of uptake in *Portunus*, higher urine/plasma ratios were found which indicates a similarity to *Carcinus* that may exist in the other species as well.

TABLE 7. LEVELS OF ^{134}Cs AND K IN PLASMA AND URINE OF THREE SPECIES OF CRAB DURING UPTAKE OF ^{134}Cs FROM ARTIFICIAL SEA WATER

(The ratios are from results calculated as per kg H_2O using a plasma water content of 92·5 % and a urine water content of 96·3 %.)

Species	Time (h)	Ratio, plasma/sea water		Ratio, urine/plasma	
		K	^{134}Cs	K	^{134}Cs
Carcinus maenas	50	1·31	0·39	1·04	0·82
	52	1·01	0·38	1·08	1·45
	120	1·07	0·60	0·96	1·31
	122	1·14	0·57	1·01	1·90
	217	1·19	0·74	0·94	1·89
	218	1·13	0·88	1·02	2·48
	1200	1·14	1·00	1·00	0·95
	1320	1·04	1·45	0·94	1·20
	1657	1·08	1·19	0·82	1·01
	1824	1·07	1·31	0·97	1·06
	1824	1·53	1·67	—	0·63
(Loss experiment)	1582	1·02	0·21	1·13	0·53
Carcinus maenas (Sea water with 50 % K)	1218	1·22	2·20	1·03	0·85
	1607	1·28	1·36	1·01	1·00
	1747	1·52	1·41	0·83	0·85
	1748	1·24	1·40	1·13	0·85
Portunus puber	957	1·39	1·29	0·76	1·27
	980	1·62	2·06	0·71	0·82
	1129	1·32	1·20	0·85	1·21
Cancer pagurus	766	1·14	1·15	0·98	1·55
	911	1·21	1·05	0·81	1·22

DISCUSSION

In the family Portunidae the species in order of the decreasing rate of accumulation of ^{134}Cs by whole animals are *Polybius* > *P. depurator* > *P. puber* > *Carcinus*. This is the order which might be expected when the habitat of each animal is considered. The rather transparent swimming crab *Polybius* would be expected to be more permeable than *P. depurator*, which would in turn be expected to be more permeable than the shore-living *P. puber* and the euryhaline *Carcinus*. However, the first three species take up ^{42}K at a very similar rate, separating them from *Carcinus* where uptake is very much slower. *Corystes* takes up ^{42}K much more rapidly than *Cancer*, yet in the one experiment which was carried out it accumulated ^{134}Cs at least as slowly. As has already been pointed out, the lack of any close relationship between the rates

at which the two ions are taken up in the different species would make it impossible to predict with any accuracy the rates of uptake of ^{134}Cs in other species on the basis of experiments with ^{42}K.

Most, if not all, of the K in these crabs is readily exchangeable with ^{42}K. Although ^{134}Cs is accumulated much more slowly than ^{42}K by whole animals the concentration factors reached at equilibrium are higher. The penetration of ^{134}Cs into the blood is much slower than the penetration of ^{42}K, and at equilibrium the concentration factor reached is rather greater than that for K while both exceed 1·0. These ions presumably enter the blood via the cells of the gills. At equilibrium the concentration factor for ^{134}Cs in the gills is about twice that for K. The higher concentration factors for ^{134}Cs in gills and blood must represent differences between the relative rates of uptake and loss of ^{134}Cs and K at the inner and outer borders of the gill cells. Webb (1940) showed that in *Carcinus* the blood K level was rather greater than that of the outside medium which indicated that some active transport of K must have taken place between the sea water and the blood. With ^{134}Cs a proportionally greater amount of active uptake must have taken place, particularly as relatively more ^{134}Cs than K is usually removed in the urine. However, active movements of both ^{134}Cs and ^{42}K are probably relatively unimportant compared with penetration of the isotopes into the blood by diffusion and exchange diffusion. No attempt has been made to measure the proportion of ^{134}Cs which penetrates by these different means. All tissues take up ^{134}Cs far more slowly than ^{42}K but the ^{134}Cs concentration factors at equilibrium exceed those for K. In *Carcinus*, tissues took up and lost ^{134}Cs in order of decreasing rate: excretory organ > hepatopancreas > muscle > vas deferens. This was probably also true of the other crabs. It appears to be a fairly general rule that muscle takes up radioactive Cs more slowly than the majority of soft tissues. For instance the scallop *Pecten irradians* showed a slower uptake of ^{137}Cs by muscle than other soft tissues and a higher activity was eventually reached (Chipman, 1960).

Mullins (1959, 1960) has attempted to explain why Rb, Cs and Na penetrate passively into frog sartorius muscle more slowly than K in terms of differences in the crystal radii of these ions. He suggests that the permeability of the muscle membrane to a given ion depends on the number of pores in the membrane which are of an appropriate size. Changes in the relative permeability of the membrane to different ions would indicate that the relative numbers of appropriate pores had changed. In support of this theory Mullins & Moore (1960) measured the fluxes of univalent thallous ions across the muscle membrane. These ions have a crystal radius which lies between those of K and Rb. The results suggested that the fibre membrane was unable to distinguish between K and Tl, in that similar fluxes were found for the two ions and at equilibrium Tl was distributed across the membrane according to a Donnan ratio similar to that for K. The distribution of K across the fibre

membrane in frog sartorius appears to be adequately explained by the theory put forward by Boyle & Conway (1941) (Conway, 1960). In its simplest form the theory supposes that a Donnan equilibrium is set up across the fibre membrane as a result of the presence of negatively charged non-diffusible material inside the cell and a high external Na concentration. Normally the permeability of the membrane to Na is lower than that to K and Cl ions which are assumed to move passively and any leakage of Na into the cell is corrected by active extrusion. This theory is supported by the fact that measurements of the actual fibre membrane potential are similar to the calculated potential difference required to maintain the observed K distribution passively. In *Carcinus* muscle Shaw (1958) compared the observed and calculated potential differences across muscle fibres and concluded that for the most part K distribution was passive. It has been shown that in *Carcinus* and other crabs tracer amounts of ^{134}Cs are distributed between tissues and blood at equilibrium in such a way that tissue/plasma ratios may be double those for K. If ^{134}Cs were also passively distributed in *Carcinus* muscle then regardless of the time taken to reach equilibrium the final ^{134}Cs muscle/plasma ratio would be expected to equal that for K but not exceed it. Relman *et al.* (1957) have discussed ways in which higher tissue/plasma values for Cs than K could be achieved in rats fed on equal amounts of these ions. The two general alternative explanations are to suppose that active transport is in some way responsible or to explain results in terms of selective adsorption within the cell. An example of the former explanation would be to suppose that in crab tissues there is a small proportion of active K uptake. The same carrier although probably having a smaller affinity for Cs than K would be responsible for some ^{134}Cs uptake. If passive uptake of ^{134}Cs was sufficiently slow then the active component could still constitute a greater percentage of the total influx than in the case of K. Provided losses were correspondingly slow a ^{134}Cs tissue/plasma ratio greater than that for K could be achieved. An alternative theory is that of Ling which bases the selective accumulation of K by cells on cation binding at fixed negative charges within the cell. The relatively higher internal level of ^{134}Cs would be explained in terms of preferential binding. The most recent evidence for this 'Fixed Charge—Induction Hypothesis' is given by Ling (1960).

Webb (1940) analysed the urine of *Carcinus* and concluded that Mg and SO_4 were selectively removed from the animal while K was retained. The urine/plasma ratios for Mg and SO_4 were respectively about 3·9 and 2·2. In order to obtain such ratios it must be assumed that either water has been reabsorbed from a urine produced initially by filtration of the blood or that these ions are secreted either in full or in addition to those in the filtered urine. A urine/plasma ratio of about 1·0 for inulin in the lobster *Homarus americanus* found by Burger (1957) under similar conditions indicates that water reabsorption is possibly not important and that these ions are secreted to a large extent.

The urine/plasma ratio for K found by Webb was about 0·78, indicating that if urine is produced initially by filtration then some K had been reabsorbed. In the present work the urine/plasma K ratio found in *Carcinus* was about 1·0, indicating neither reabsorption nor secretion but ratios as high as 2·48 were obtained for ^{134}Cs which are presumably the result of some secretion. Under circumstances where equilibrium was approached, this ratio for ^{134}Cs fell to about 1·0 and in some cases to a lower level which suggested reabsorption. The reason for the fall in ^{134}Cs urine/plasma ratios as equilibrium was approached did not appear to be a result of long starvation and no other satisfactory explanation has been found. It is possible that after the period required to attain equilibrium the animal in some way becomes adapted to Cs ions and ceases to excrete them preferentially.

Robertson (1939) showed that in *Cancer* the urine/plasma ratio for K was 0·81. In the present work a mean value of 0·89 was found while that for ^{134}Cs at equilibrium was 1·38. This suggests that K may be reabsorbed from primary filtered urine while ^{134}Cs is secreted into it. Similarly, in *P. puber* the mean urine/plasma ratio for K was 0·77 while that for ^{134}Cs at equilibrium was 1·1. As in *Carcinus* the cells of the excretory organs of *Cancer* and *P. puber* have higher tissue/plasma ratios for ^{134}Cs than K. When a urine/plasma ratio of 1·0 is found for K or ^{134}Cs then presumably excretory cells in contact with both blood and urine are maintaining similar equilibria across both cell walls. Whether the cells absorb or secrete K or ^{134}Cs giving urine/plasma ratios less than 1·0 will probably depend on the two walls of the excretory cells behaving differently from each other. The cells lining the proximal tubules of *Necturus* kidney have definitely been shown to be asymmetric (Whittembury, 1960) and this may also be the case in crabs.

A feature of ^{134}Cs absorption from food was the rapidity and completeness with which it took place. It has not definitely been shown which part of the gut is responsible for the absorption of ions but the rapid increase in the activity of the hepatopancreas after a meal suggests that it may be the main site of ^{134}Cs uptake. The isotope probably reaches the blood and then the other tissues by some absorption through the walls of the rest of the gut as well.

I should like to thank Mr L. G. Hummerstone for his help with this work which was supported by the United Kingdom Atomic Energy Authority.

SUMMARY

The accumulation of ^{134}Cs and ^{42}K from artificial sea water has been followed in crabs of the following species, viz. *Carcinus maenas*, *Portunus puber*, *P. depurator*, *Polybius henslowi*, *Cancer pagurus* and *Corystes cassivelaunus*. In all cases ^{134}Cs is taken up far more slowly than ^{42}K but at equilibrium a

higher concentration factor is attained by ^{134}Cs. In *Carcinus* a value of about 8 is found for ^{134}Cs and about 4 for ^{42}K. Uptake of ^{134}Cs in *Carcinus* is not affected by feeding on inactive food over a period of 1000 h. The relationship between the rates of uptake of the two isotopes is not the same in the different species and it is concluded that it would not be possible to predict with any accuracy rates of uptake for ^{134}Cs in other species of crabs from the results of short ^{42}K experiments.

Uptake of ^{134}Cs was followed in the blood and tissues of *Carcinus* and *Cancer*, and muscle is the principal limiting factor in the attainment of equilibrium by whole animals. Uptake of ^{42}K by tissues has also been followed and in all species nearly all the inactive K appears to be readily exchangeable. At equilibrium in all species plasma/sea water and tissue/plasma ^{134}Cs ratios nearly always exceed those for inactive K. The tissue/plasma ^{134}Cs ratios in *Carcinus* are about twice those for K and the significance of this has been discussed.

Loss of both isotopes in whole *Carcinus* is a slower process than uptake. Isotope accumulation takes place mainly across the body surface in unfed animals, but uptake of ^{134}Cs from food is very rapid and complete and would considerably enhance the attainment of equilibrium in a natural environment.

Selective excretion of ^{134}Cs relative to K has been found in *Carcinus*, *Cancer* and *Portunus puber*.

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THE BEHAVIOUR OF *POLYDORA CILIATA* (JOHNST.). TUBE-BUILDING AND BURROWING

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(Text-figs. 1–10)

Polydora ciliata (Johnst.) is a common but inconspicuous polychaete which burrows in a variety of rocks varying in hardness from the Devonian limestone found at Plymouth to the softer chalk of the Isle of Wight and the London clay at Whitstable on the north Kent coast. It can be a troublesome pest of oysters (McIntosh, 1908; Lunz, 1941; Korringa, 1951), perforating the shell with its galleries. All these materials contain calcium carbonate, the worms being apparently unable to penetrate granites and silicious rocks not containing this substance. McIntosh (1915) found worms burrowing in the non-calcareous shales at St Andrews and I have found them in wooden piles along the Kent coast.

No recent account exists in the literature of either the anatomy or biology of *P. ciliata*. Many of the earlier accounts (Dalyell, 1853; McIntosh, 1915) appear to be in need of revision, and knowledge of the feeding and tube-building mechanisms, life span, fecundity and growth rates is fragmentary.

The burrow

The burrow (Fig. 1 A) is a U-shaped cavity in the substratum, the central part being filled with a partition of loose material cemented together by a mucous secretion of the worm. Under a dissecting microscope this partition usually presents a layered appearance. In burrows in pure limestone the central partition is usually made up of silt and other debris accumulated from outside the burrow, but in clay it contains sand grains and particles of silt which may represent material eroded by the worm in the process of burrowing. The layered appearance of the central partition may mean that deepening of the burrow occurs only at certain times, each thin layer representing a mucous cover over the recently excavated material.

The two limbs of the burrow are lined by a tube composed of mucus and fine sand particles, and this has a smooth mucus lining (Fig. 1 B). The tube, which projects above the substratum to a height of 0·5 mm, is complete but for a small section at the bottom of the burrow where deepening occurs. In

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the laboratory the worms can build up their tubes to a height of 3 mm but this does not happen on the shore, the tubes being eroded by wave action.

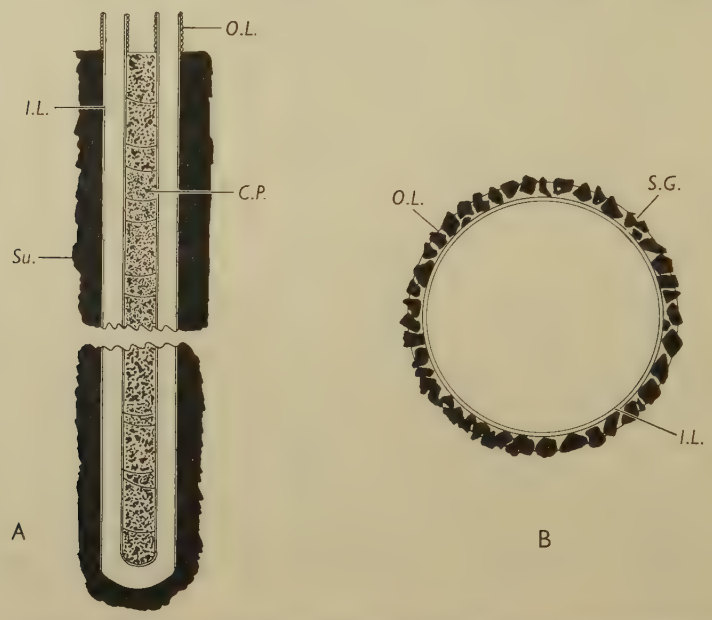

Fig. 1. *Polydora ciliata.* A, Longitudinal section of burrow. B, Transverse section of tube. *C.P.*, central partition; *I.L.*, inner lining; *O.L.*, outer layer; *S.G.*, sand grain; *Su.*, substratum.

Collection of material

Whilst tube-building *P. ciliata* lies with its head just visible inside the rim of the tube. The palps emerge from the tube and are applied to the substratum with the ciliated groove downwards. This differs from the position adopted when feeding, the palps then performing lashing movements by which particles suspended in the water are collected. The palps are a pair of tapering cylindrical structures arising from the dorsal side of the peristomium. Along their inner surface is a ciliated groove (Fig. 2) which runs from the distal end of the palp to a short distance from the proximal end. The cilia beat towards the head and particles of both food and tube-building material are caught up by them and wound into a mucus string. The tips of the palps are forced among the bottom debris and sand grains are dislodged and carried posteriorly toward the mouth. The ciliated groove ends short of the base of the palp and the mucus string is forced out and met by the prostomium which bends towards it. The string is picked up by the densely ciliated ventral epithelium of the prostomium (Fig. 3, *cil.ep.*) and is carried towards the mouth.

As the ventral ciliated epithelium of the prostomium passes into the peristomial region it develops a ciliated fold on each side of the median ridge

(Fig. 4A–E). The outer edges of the epithelium are joined to the inner edges of the lateral lips and in the posterior peristomial region, where the lips meet and join mid-ventrally to form the mouth, the non-ciliated epithelium of the lips still connects the edges of the lateral ciliated folds to form the floor of the anterior part of the gut. Thus, from the mouth to the second segment, the

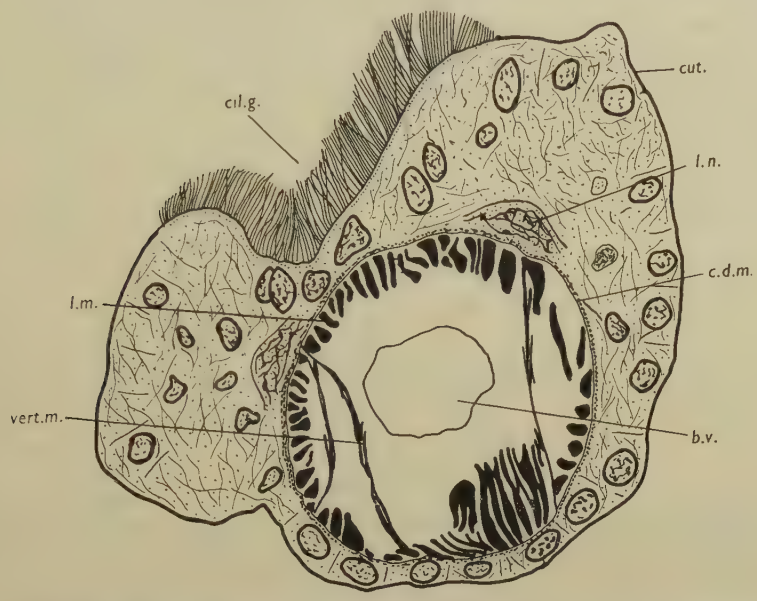

Fig. 2. *Polydora ciliata*. Transverse section of palp. *b.v.*, blood vessel; *cil.g.*, ciliated groove; *c.d.m.*, circular and diagonal muscle fibres; *cut.*, cuticle; *l.m.*, longitudinal muscle; *l.n.*, longitudinal nerve; *vert.m.*, vertical muscle fibres.

floor of the gut is occupied by a non-ciliated gutter (Fig. 4E). Observations of the worm when tube building show that material is diverted between the lips into the region of the non-ciliated gutter, whereas when feeding, particles pass unhindered down the gut.

There is some indication that *P. lignii* has the ability to distinguish particles by their chemical nature (Mortensen & Galtsoff, 1944) but the constant presence of small sand grains in the gut of *P. ciliata* suggests that the size of the particle might form the basis on which selection of material for tube building or food is made. To test this a group of *P. ciliata* were removed intact in their tubes from the shore to the laboratory and their faecal pellets were allowed to accumulate. These were then removed and the largest linear dimensions taken from a sample of 200 sand grains. The dimensions of a similar sample of diatom frustules were also taken. The worms were allowed to build up their tubes until they projected some 3 mm above the surface of the clay. The projecting tubes were then cut off and boiled in caustic potash

to remove the mucoprotein binding the grains together. The linear dimensions of a similarly sized sample of sand grains was taken from the tubes.

It can be seen (Fig. 5) that the majority of particles passing through the gut are not above 0·03 mm whilst those used in construction of the tube are mostly above 0·05 mm. Selection usually occurs between these two limits. Some overlap is to be expected from the arbitrary measurements taken from the samples. The largest particles entering the gut might be long and narrow, their admission depending on their orientation. Again, the smallest particles from the tube might originate from some larger mass that was stuck on the tube as a whole.

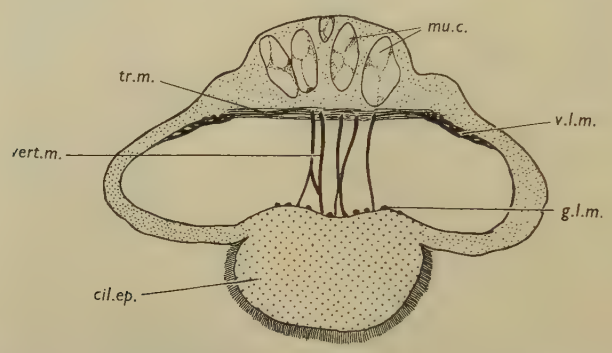

Fig. 3. *Polydora ciliata*. Transverse section of prostomium. *cil.ep.*, median ciliated ridge; *g.l.m.*, longitudinal muscle of gut; *mu.c.*, mucous cells; *tr.m.*, transverse muscle fibres; *v.l.m.*, ventral longitudinal muscle; *vert.m.*, vertical muscle fibres.

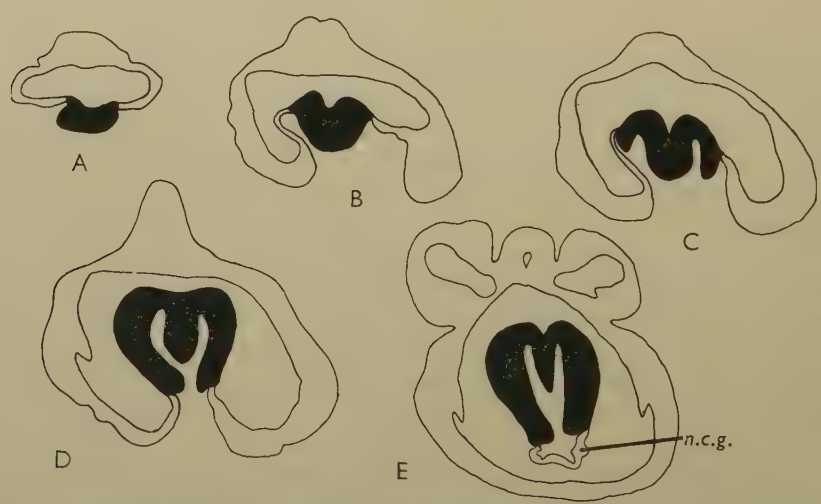

Fig. 4. *Polydora ciliata*. A–E. Camera lucida drawings of sections taken at progressively posterior levels through the prostomium and peristomium. Ciliated epithelium, black. *n.c.g.*, non-ciliated gutter.

A mechanism of particle selection is suggested in Fig. 7A–E. At the point where the lateral ciliated folds are given off from the median ridge on the ventral side of the peristomium, particles too large to pass between the lateral fold and the median ridge would be forced to travel along the outer edge of the ciliated epithelium and would end up in the non-ciliated gutter. Those small enough to pass between the folds would pass unhindered down the gut. Such a mechanism would allow particles collected by the palps to be

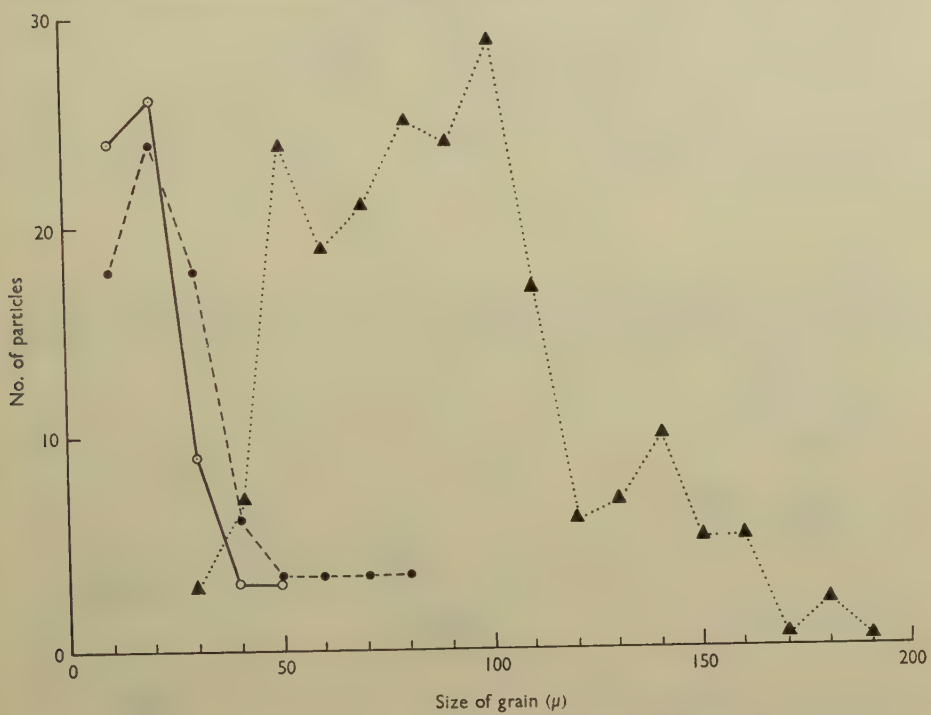

Fig. 5. *Polydora ciliata*. Analysis of sand grains and diatom frustules from tube and faeces. Continuous line, sand from gut; broken line, diatoms from gut; dotted line, sand from tube.

sorted into two size groups by a mechanism somewhat similar to that described for *Sabella pavonina* (Nicol, 1931). In this species they are sorted into three size groups between the inclined walls of the gill folds. The finest particles are passed direct to the mouth whilst the medium sized particles are used in the construction of the tube. The largest particles are rejected.

Observations on particle selection are difficult to make and narcotized animals examined under the microscope do not accept particles introduced on to the palps since the mobility of the prostomium is an essential part of the collecting process. Sections of the worms used in the selection experiments were cut and stained. The specimens were first narcotized and then fixed in

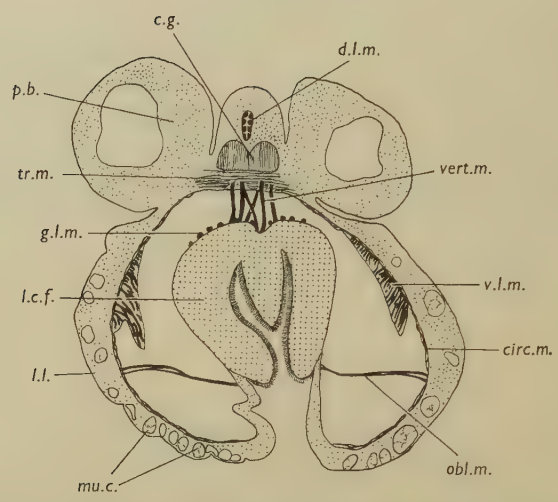

Fig. 6. *Polydora ciliata.* Transverse section of peristomial region. *l.c.f.*, lateral ciliated fold; *circ.m.*, circular muscle fibres of lips; *c.g.*, cerebral ganglia; *d.l.m.*, dorsal longitudinal muscle; *g.l.m.*, longitudinal muscle of gut; *l.l.*, lateral lips; *mu.c.*, mucous cells; *obl.m.*, oblique muscle; *p.b.*, palp base; *tr.m.*, transverse muscles; *vert.m.*, vertical muscle.

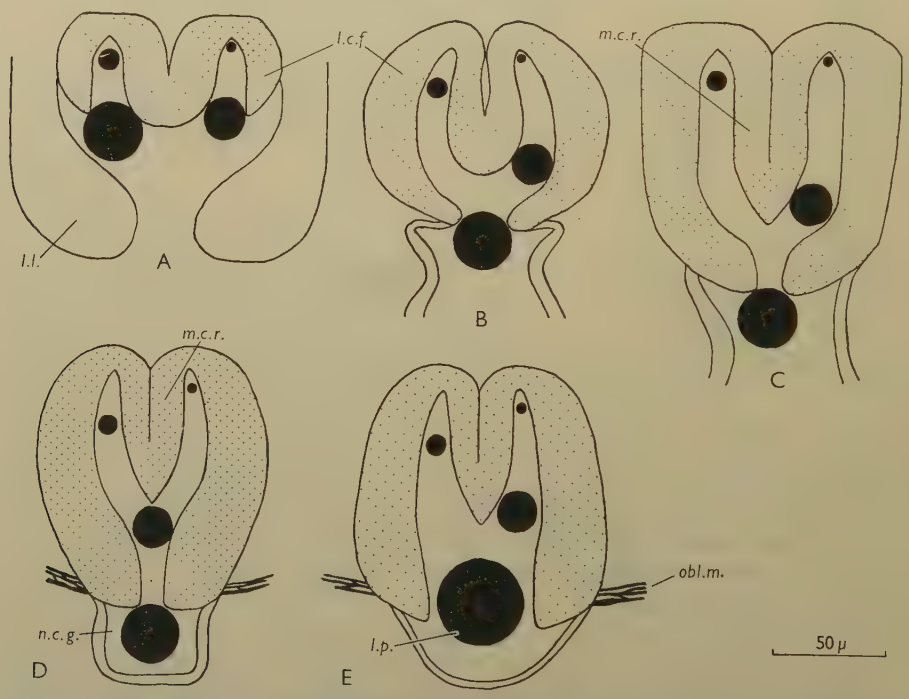

Fig. 7. *Polydora ciliata.* Suggested particle selection mechanism. *l.l.*, lateral lips; *l.c.f.*, lateral ciliated folds; *l.p.*, large particle; *m.c.r.*, median ciliated ridge; *n.c.g.*, non-ciliated gutter; *obl.m.*, oblique muscle.

Gilson's fluid. In this way shrinkage was kept to a minimum. The distance between the lateral ciliated folds and the median ridge, which is critical in deciding the point where separation of the two groups of particles is made, was measured and found to be 0·032 mm. This corresponds closely to the size at which division is made in nature.

In the laboratory worms will accept food particles larger than those taken under normal conditions. Examination of the muscular anatomy of the peristomium shows how this might be achieved. Contraction of the oblique muscle (Fig. 6, *obl.m.*) which crosses the coelomic cavity of the lateral lips to the edge of the ciliated epithelium, and relaxation of the vertical muscle fibres which pass from the dorsal body wall to their insertion above the median ridge, against a positive pressure of the coelomic fluid, would allow the groove between the ciliated epithelia to widen and a larger sized particle to be accepted (Fig. 7E).

Construction of the tube

In the normal pattern of behaviour, tube-building alternates with periods of feeding. In the laboratory, tube-building behaviour can be initiated by adding a suspension of clay particles to the water artificially circulating in the dish. It may also occur spontaneously in worms living in filtered sea water that is not in movement. Under these conditions the animals eventually become quiescent except for intermittent excursions to the mouth of the tube to sample the water.

When adding material to the tube the worm crawls forward until its head emerges from the end. The lateral lips then diverge and the sand grains, which have been diverted into the non-ciliated gutter (see above), are pushed forward between them (Fig. 8A). The worm then bends its head towards the side of the tube and the sand grain is placed on the rim. It then retreats into the tube only to re-emerge immediately and cement the grain more firmly in position, pressing the ventral body wall of the first and second segments over the region where the sand grain was placed. The epithelium of both the lips and the ventral body wall contains large mucous cells which cover the grains with a layer of secretion. The mucus hardens rapidly in sea water and fixes them securely. The pressing may be repeated a second time. The worm retreats into the tube whilst more material is collected by the palps and the cycle is then repeated. In subsequent cycles, before placing more material on the rim of the tube, the body is rotated through a small angle so that the grains are placed by the side of those deposited in the previous cycle. The direction of turn appears to be constant in any one phase of tube-building activity but may differ in following phases. In this way the tube is built up evenly on all sides.

The worm emerges from the tube until the seventh chaetigerous segment is just visible, the body is held quite straight with the palps in front of the

head and immobile (Fig. 8B). The worm then rotates its body through 360° about its longitudinal axis, first in one direction and then in the other. Whilst it is turning, the notopodial chaetae are protracted and retracted in a 'treading' motion against the wall of the tube. This treading activity is frequently followed by four rapid protrusions of the head from the tube,

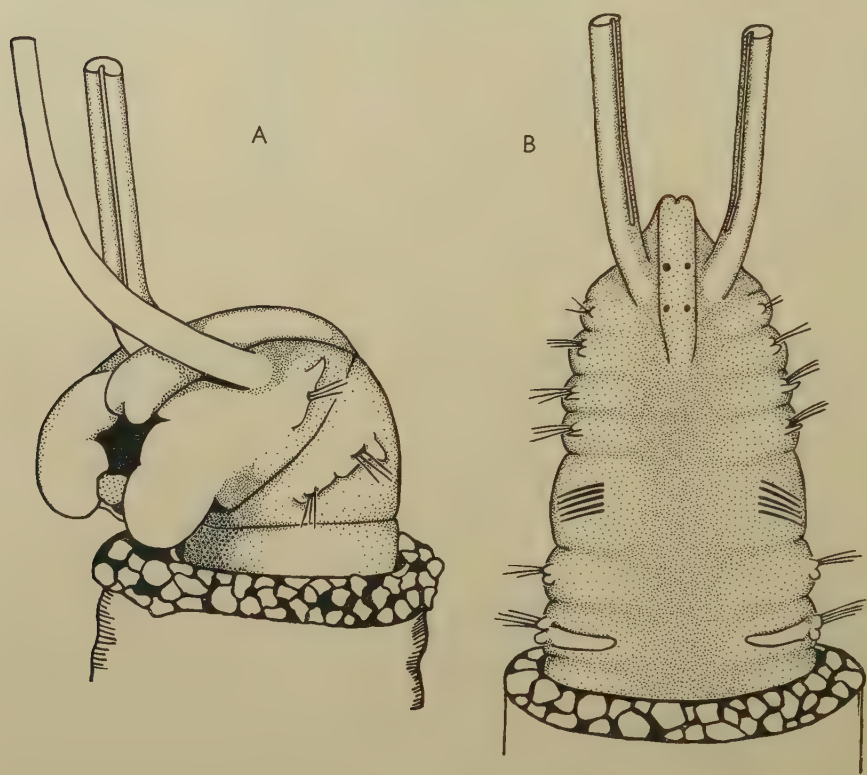

Fig. 8. *Polydora ciliata.* A, Placing sand grain on rim of tube. B, Tube lining position.

identical to those in which a sand grain is being placed but no grain being deposited. Between each excursion the body is turned through one right angle so that all four sides of the rim are touched in turn by the lateral lips. In this way the worm probably senses any irregularity of the rim of the tube.

The significance of this last pattern of activity becomes clear when one examines the structure of the tube and the anatomy of the thoracic segments. It is in the seventh segment that the segmental mucus glands, the 'poches glandulaires' of Fauvel (1927), first appear. These glands (Fig. 9, *s.m.g.*) are found protruding into the ventral coelom in a variable number of segments after and including the seventh. Each gland consists of six or seven large

mucous cells so closely apposed as to resemble a single transparent vesicle. From each cell an intra-cellular duct passes behind the neuropodial chaetae to emerge on the ventro-lateral body wall in a small papilla. There are a group of the papillae on each side of the mid-line in each segment that bears the glands.

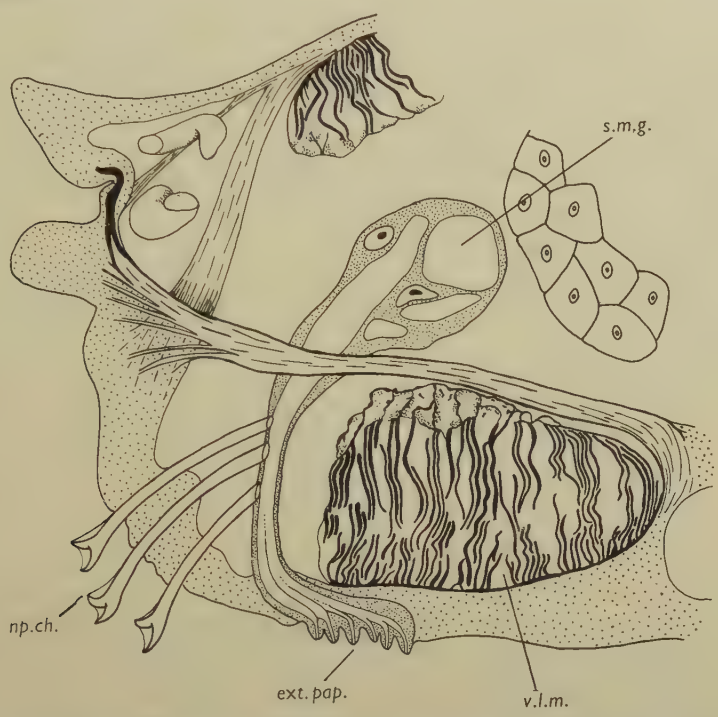

Fig. 9. *Polydora ciliata*. Transverse section of trunk segment posterior to branchia. *ext.pap.*, external papillae of gland; *np.ch.*, neuropodial chaetae; *s.m.g.*, segmental mucus gland; *v.l.m.*, ventral longitudinal muscle.

It seems that after the worm has added a new length to the tube, the inner lining is still rough. The worm then emerges as far as the seventh segment and rotates its body, spreading the secretion of the segmental mucus glands on the unlined portion of its tube and working it into the outer layers with its chaetae. The secretion hardens rapidly in sea water, forming the inner lining to the tube. The staining reactions of the segmental mucus glands and tube lining with alcian blue suggest an acid mucopolysaccharide similar to that identified in the glands and tubes of other polychaetes (Ewer & Hanson, 1945).

Excavation of the burrow

Theories invoking both chemical solution and mechanical abrasion have been advanced to account for the method by which *P. ciliata* penetrates its

various substrates. Chemical penetration was thought to be achieved by means of an acid secretion which would dissolve calcium carbonate, but no acid has yet been identified in polychaetes. Söderström (1920, 1923) at first considered that boring was effected by an acid secretion of the segmental mucus glands combined with the mechanical action of the chaetae of segment five. In the later paper he decided that the uncini of the fifth segment were used only for attachment and played no part in the boring process. Those postulating a mechanical method of burrowing have called attention to the uncinate chaetae of the fifth segment which are thicker and larger than those

TABLE 1. THE ARRANGEMENT OF SEGMENTAL CIRCULAR MUSCLES IN *POLYDORA CILIATA*

Segment	No. of fasciculi	No. of fibres/ fasciculus
1	2	6
2	2	6
3	2	12
4	2	20
5	6	20–70
6	8	20–80
7	3	60–70
8	3	20
9	3	20
10	2	20
11	2	20
12	2	20
13 and post	1	20 or less

of the other segments. An acid would be of little use in the penetration of wood, and the fact that they can only penetrate the outer layers where the wood is soft and rotten suggests that they excavate the burrow with their chaetae. A recently metamorphosed worm of 30 segments, removed from its primary tube and placed on the surface of a piece of clay, adopted a position in which the chaetae on one side of the fifth segment were used as a fixed point whilst those of the other side were flexed ventrally and then flicked violently upward (see similar observation of a newly settled worm in Wilson, 1928, pp. 583–4). This is essentially the movement that would be used in mechanical excavation of the burrow and was observed to dislodge some small sand grains from the surface. The entire musculature of the fifth segment, apart from the longitudinal muscles, is devoted to producing a dorso-ventral movement of the chaetae. The dorsal circular muscle fibres (Fig. 10, *d.lev.m.*) are inserted on the dorsal border of the chaetal sac where it meets the body wall, and its action is reinforced by the dorsal circular muscles of segments six and seven which pass forwards to the same insertion. The circular muscles of *Polydora ciliata* resemble those of the errant polychaetes in being drawn together dorsally in each segment into fasciculi. It can be seen from Table 1 that both the number of fasciculi and the number of fibres in each fasciculus is greater in segments five, six and seven than in any other segment.

The chaetae of the fifth segment are not used in normal locomotory movements and the nature of the muscle supply together with the orientation of the chaetae in the sac points to the fact that they are entirely devoted to producing a powerful upward flick.

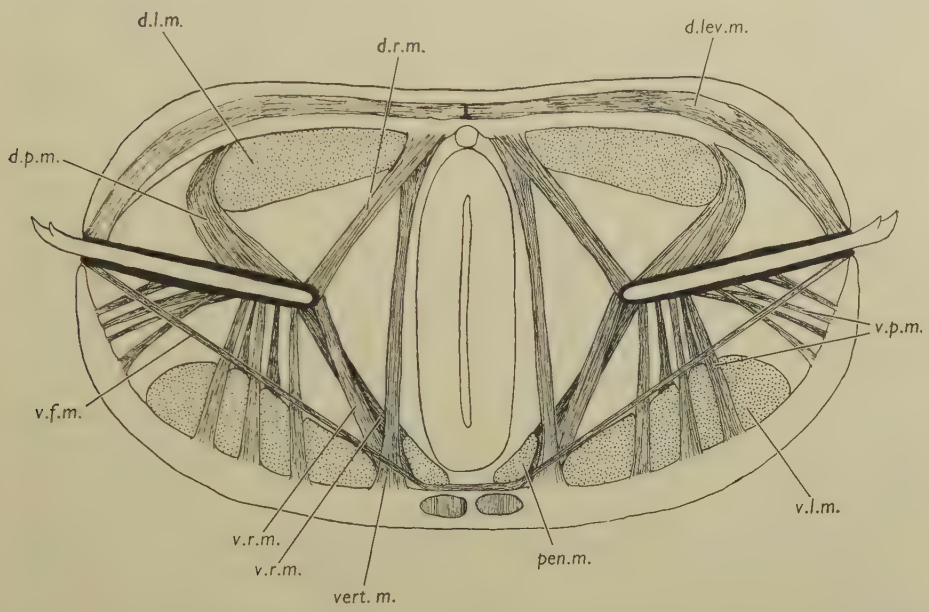

Fig. 10. *Polydora ciliata.* Transverse section of fifth segment showing muscle supply to uncini. *d.lev.m.*, dorsal levator muscle; *d.l.m.*, dorsal longitudinal muscle; *d.p.m.*, dorsal protractor muscle; *d.r.m.*, dorsal retractor muscles; *pen.m.*, pennate muscles; *vert.m.*, vertical muscles; *v.f.m.*, ventral flexor muscle; *v.l.m.*, ventral longitudinal muscle; *v.p.m.*, ventral protractor muscle; *v.r.m.*, ventral retractor muscle.

On the other hand, it seems unlikely that the relatively delicate chaetae of *Polydora* could produce cavities $26 \times 3 \times 1$ mm in hard limestone. In order to test (rather simply) the effect of mechanical abrasion by a chaeta, a neuropodial chaeta of *Aphrodite aculeata* was mounted vertically against a block of limestone and rotated at 85 rev/min for 112 h under a pressure of 10 g. At the end the rock showed no sign of abrasion though the chaeta was shorter by 0·04 mm. Such evidence suggests that the tanned protein which forms the chaetae of polychaetes (Ebling, 1945) is too soft a material to be alone responsible for the excavation of the burrow in limestone. Comparison of the tips of the uncini of the worms boring in clay at Whitstable with those boring in limestone at Plymouth shows that whilst those of the Plymouth worms are a little more rounded there is comparatively little more wear. Possibly the galleries in limestone are occupied and deepened by succeeding generations, but this does not occur at Whitstable where old burrows are rapidly filled in.

Though it seems that *P. ciliata* in fact penetrates some materials by the mechanical action of the chaetae, yet the penetration of limestone must still be accounted for. Several authors (List, 1902; Russell-Hunter, 1949; Yonge, 1955) suggest the presence of an acid secretion to assist organisms, particularly molluscs, to penetrate calcareous rocks, but they do not specify what acid. More recently, Thompson & Slinn (1959) have demonstrated secretions of opisthobranchs which they believe to be a mixture of sulphuric and hydrochloric acids. Mineral acids will be effective in borers only if applied directly to the area being eroded, to the exclusion of sea water, as the alkali reserve of the latter would rapidly neutralize the small quantities of acid produced. The inside of the burrow of *P. ciliata* does not have the same appearance as limestone that has been treated with dilute mineral acid. Acid produced a smooth polished surface whereas the walls of the burrow are fairly rough.

Solution of the calcium carbonate by the sea water charged with the respiratory carbon dioxide could only play a very small part in burrowing, as the rate of solution is extremely slow. Hall described in 1935 how calcium ions could be eliminated whilst still retaining them in solution. This effect, known as sequestering, finds use in zoology in the decalcification of delicate material for section cutting. Certain phosphates, notably sodium hexametaphosphate bring about quite rapid solution in this way and might be produced by a biological system.

It is interesting to note that, in those organisms studied, the ability to achieve penetration by chemical means is frequently associated with the presence of mucus glands in the region producing the secretion. The mantle lobes of *Hiatella* (see Russell-Hunter, 1949) and *Lithophaga* (see Yonge, 1955) both have glands giving a mucous-staining reaction in the region producing the chemical solvent. The ventral epithelium and segmental mucus glands of *P. ciliata* have an abundance of cells staining with alcian blue, which indicates an acid mucopolysaccharide. Simkiss & Tyler (1958) have demonstrated how acid mucopolysaccharides in the spongy layer of the egg shell of the hen may act as a chelating agent for metallic ions, calcium in particular. There seems no reason to suppose that the mucous secretions of *P. ciliata* and other rock-boring organisms could not behave in a similar way to bring about solution of calcareous substrates.

I should like to record my thanks to Prof. J. E. Smith, F.R.S., for his advice and criticism in all stages of this work, to Prof. G. E. Newell and Prof. J. E. Webb for reading the typescript, and to the Director of the Marine Biological Association for facilities afforded whilst I was working at the Plymouth Laboratory.

SUMMARY

Polydora ciliata is a spionid polychaete found below mid-tidal level burrowing in a variety of rocks all of which contain calcium carbonate. It can also penetrate some non-calcareous materials such as rotten wood. It excavates a U-shaped burrow which it lines with a tube composed of mucoprotein and sand grains. This tube has a smooth inner lining of mucoprotein. Both food and tube-building material is collected by the palps and it is suggested that discrimination is made chiefly on size of the particle. Selection is made between the limits of 0·03–0·05 mm, the smaller particles passing down the gut whilst the larger are used in construction of the tube. A particle selection mechanism is suggested. The function of the segmental mucus glands is to provide the inner lining of the tube.

The problem of chemical penetration of rocks by animals is discussed. *P. ciliata* uses both mechanical and chemical methods. No acid has been identified, and the use of a sequestering or chelating agent linked with the biochemistry of mucus is suggested.

Since preparing this paper for the press, my attention has been drawn to a paper by Hempel (1957). In a general account of the morphology and tube-building of four members of the genus *Polydora* and *Pygospio elegans*, she describes the collection of food and tube-building material in *Polydora ciliata*. Her observations are in agreement with my own, although she does not analyse the tube-building behaviour in detail. She found that sand grains between 0·2 and 0·05 mm were preferred in constructing the tube, but concludes that the texture and weight are more important than the size of the particle. She did not examine the contents of the faecal pellets for inorganic particles, the presence of which suggests that size is the principal factor in selection. In a survey of the types of substratum chosen by *P. ciliata* in which to burrow, she found that in the molluscan shell the calcite layer is preferred to the aragonite layer because of its softer nature. Boring, which in the recently metamorphosed worm may be assisted by an acid secretion, is thought in the adult worm to be achieved by the mechanical action of the uncinate chaetae of the fifth segment. Evidence for this is principally in the presence of scratches on the wall of the burrow and wear on the chaetae, but again no attempt was made to determine the degree of hardness of the chaetae with respect to the material they erode.

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THE ULTRA-VIOLET ABSORPTION OF SEA WATER

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The Plymouth Laboratory

(Text-figs. 1–3)

The intensity of submarine daylight is of such importance to the growth of marine plants that much attention has been given to measurements of the absorption of solar radiation by sea water. It has long been known that absorption is least in the blue green in the visible spectrum, and Tsukamoto (1927) showed that the absorption of ultra-violet radiation increased very steeply between 300 and 200 mμ. Kalle (1937) showed that absorption in the blue increased in coastal waters, and ascribed the effect to increased absorption of indeterminate organic materials which he thought to be similar to the 'humic acids' of river and lake waters, and which he named 'Gelbstoff'. Joseph (1949) used a submersible photometer and was able to extend measurements to about 367 mμ, just outside the visible region, and showed that the absorption of 'coastal' waters was enhanced at this wave-length. Baltic Sea water of low salinity was in fact much less transparent than water from the North Sea. Jerlov (1950) further extended measurements to 310 mμ and revealed even greater differences between different sea waters. In the eastern Mediterranean for example, where the water was of clarity comparable with that of the Sargasso Sea, the loss per metre at 310 mμ was 14%, compared with 90% in the Skagerrak. At 375 mμ the eastern Mediterranean water absorbed only 5% per metre so that in such clear water biologically important radiation might, in his opinion, penetrate to about 20 m. Jerlov (1951) showed that a considerable range of sea waters could be classified by comparison of their absorption spectra, the distinctions being greatest at short wave-lengths. Scattering by particulate matter contributed materially to the attenuation of the light beam in Jerlov's underwater apparatus and was appreciable at the shorter wave-lengths. Lenoble (1956*b*) has measured the *in situ* absorption of ultra-violet radiation down to 318·5 mμ, at various stations in the Mediterranean, and has also (1956*a*) measured the absorptions between wave-lengths of 250 and 400 mμ, of solutions of the salts which may be used to constitute artificial sea water.

On repeating these latter measurements of Lenoble, and extending them to 200 mμ, we have found a big difference between the absorption of sea water and that of its component salts, confirming a finding by Chanu (1959).

We considered therefore that further study might be interesting both as a measure of a physical property characterizing different sea waters and as a guide to their chemical composition.

We are grateful to the Director and staff of the National Institute of Oceanography for allowing one of us (F.A.J.A.) to collect samples from R.R.S. 'Discovery II', and to Mr E. I. Butler for help in collecting samples. One of us (G.T.B.) holds an International Paints Research Fellowship and his thanks are due to Mr O. D. Hunt for permission to engage in this work.

APPARATUS AND METHODS

Measurements were made, usually at intervals of 10 mμ, between 200 and 400 mμ with a Unicam S.P. 500 spectrophotometer and are recorded as the instrument readings of extinction values. Properly, the quantity measured is Absorbancy (As), where $As = \log_{10} I_0/ I$, I_0 and I being the intensities of the light beam entering and leaving the cuvette containing the sample. A 10 cm cuvette with silica windows was used and, to avoid doubt as to the purity and consistency of a liquid medium, all measurements were made with air as the reference path. There is therefore a small correction necessary for loss of light by reflexion at the end windows. An approximate direct measurement gave this as about 0·06 at 200 mμ decreasing to 0·03 at 400 mμ, these values should be subtracted from the measurements reported.

The observations must be regarded as relative, as indeed must most spectrophotometric measurements (Ewing & Parsons, 1948; Goldring, Hawes, Hare, Beckman & Stickney, 1953). We know that below 230 mμ our measured absorbancies are low. The instrument is an early model with natural quartz optics and the hydrogen discharge lamp had lost some emission, requiring slit widths greater than 1 mm. These shortcomings, by broadening the effective band width and decreasing the short wave-length intensities relative to that of scattered light, combine to give low readings particularly when, as here, the sample absorbancy is high. It is difficult to measure such errors directly, but we have been able (using sodium nitrite solutions of approximately unit absorbancy) to compare our instrument with a new one with synthetic quartz optics. The absorbancies on the older instrument, at 200, 210, 220 and 230 mμ were respectively 40, 80, 85 and 95 % of those found on the new one. The sharp loss below 210 mμ has caused a spurious maximum to appear at this wave-length in some of the curves, but having drawn attention to this we think it preferable to present our observations with no attempt to apply what would in any case be arbitrary corrections. The errors of the instrument seem to have altered very little in the course of the experiments since slit widths for given sensitivity at short wave-lengths have increased by only about 5 %.

Glassware was acid cleaned and well washed with distilled water before use. Water for spectrophotometric measurement and for making artificial sea water was twice distilled in glass (the second time over alkaline permanganate) and then passed through a column of active charcoal (Messrs Sutcliffe Speakman's Grade 315). Further attempts at purification failed to reduce the ultra-violet absorption of this water, which was assumed to contain a negligible amount of impurities.

The artificial sea water was made from analytical grade reagents to the hypothetical formula of Lyman & Fleming (1940). The sodium chloride and sulphate (the major constituents), and the potassium chloride were heated to redness before use to destroy organic impurities. The other salts would have decomposed on heating.

Sea-water samples were collected in either 25 l. glass carboys or 10 l. Pyrex bottles and were usually filtered within 36 h through well washed membrane filters or porcelain candles of average pore diameter 1 μ. Experience with these filtration methods has shown that the amount of particulate matter passing the filters is small (usually less than 0·1 mg/l.) and that the filtrates are comparable as regards Tyndall effect with water freshly distilled and collected with precautions against dust (Armstrong & Atkins, 1950; Armstrong, 1958).

We think that the contribution of light scattering by particles to the absorbancy of these filtered samples is negligible.

RESULTS

The absorption spectra of purified water, artificial sea water, and a fairly typical sample of English Channel surface water are shown in Fig. 1. Below about 240 mμ, about half the absorbancy of this natural sea water is accounted for by the inorganic constituents.

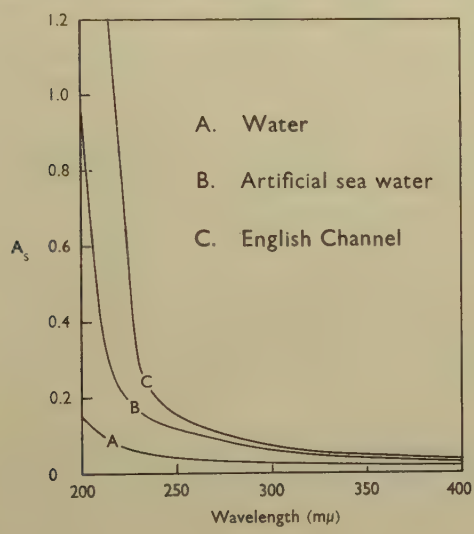

Fig. 1

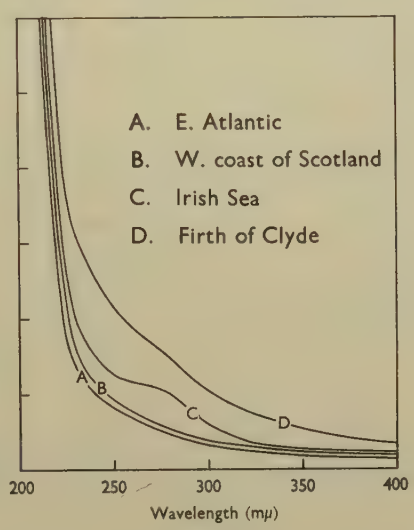

Fig. 2

Fig. 1 (left). Absorbancy against air. A, purified water; B, artificial sea water; C, English Channel surface water.

Fig. 2 (right). Absorbancy against air. A, 46° 30′ N., 8° 00′ W., May 1960. B, 58° 10′ N., 8° 33′ W., June 1960. C, 54° 28′ N., 5° 22′ W., June 1960. D, 55° 45′ N., 4° 54′ W., April 1960.

Marked differences were found between sea-water samples of different origin, greater absorbancies being observed in coastal waters. This is shown in Fig. 2, in which three surface samples of coastal waters are compared with one from the north-east Atlantic. The high absorbancy of the Clyde water is very striking. The sample was taken in April at the time of a heavy diatom

outburst and the filtered water, viewed in a 50 cm column, was visibly yellow. A good example of the geographical variation is given by the figures of Table 1. Samples were taken at 10-mile intervals on the 4° W. meridian, in the English Channel between the French and English coasts, and absorbancies recorded, at 220 mμ. The increases near the coasts are well marked.

Ultra-violet absorption has been found to vary with depth, and Table 2 gives measurements on samples from various depths at stations in the north-east Atlantic Ocean. The surface samples have relatively greater absorbancies above 235 mμ, and the deep water samples greater values below this wavelength.

TABLE 1. ULTRA-VIOLET ABSORBANCY OF SEA WATER FROM 10 M DEPTH AT POSITIONS ON 4° W. MERIDIAN ACROSS ENGLISH CHANNEL 22 FEBRUARY 1961

Latitude (N.)	48° 50′	49° 00′	49° 10′	49° 20′	49° 30′	49° 40′	49° 50′	50° 00′	50° 10′
Distance from French coast (km)	13	31	50	69	87	106	124	143	161
Distance from English coast (km)	163	145	126	107	89	70	52	33	15
A_s 10 cm, 220 mμ	0·70	0·68	0·66	0·65	0·64	0·63	0·63	0·65	0·74

TABLE 2. ULTRA-VIOLET ABSORBANCY OF SEA WATER FROM VARIOUS DEPTHS AT TWO POSITIONS IN NORTH-EAST ATLANTIC OCEAN

Position ...	46° 30′ N., 8° 00′ W., 6 May 1960			62° 41′ N., 15° 02′ W., 11 June 1960					
Depth (m)...	0	1870	4500	0	100	400	800	1200	1810
Wavelength (mμ)									
220	0·56	0·91	0·94	0·65	0·79	0·81	0·89	0·92	0·88
230	0·27	0·34	0·31	0·27	0·27	0·27	0·28	0·29	0·29
240	0·20	0·20	0·16	0·18	0·15	0·15	0·15	0·16	0·16
250	0·16	0·13	0·11	0·15	0·11	0·11	0·11	0·11	0·12
260	0·13	0·10	0·08	0·13	0·09	0·09	0·09	0·09	0·10
280	0·10	0·08	0·07	0·10	0·06	0·06	0·06	0·06	0·07
300	0·07	0·06	0·05	0·07	0·05	0·05	0·05	0·05	0·05
400	0·03	0·03	0·03	0·04	0·03	0·03	0·03	0·03	0·03

Surface, 10 and 50 m samples from a position in the English Channel were examined monthly during the period April 1959 to November 1960, and the absorbancies at 220 and 250 mμ are shown in Fig. 3. There are considerable irregularities and perhaps a slight increase in summer. Higher absorbancies often coincided with the small decreases in salinity and increases in silicate which indicate increased admixture of land drainage water. There was a slight tendency for the upper samples to have higher absorbancies than the deeper ones.

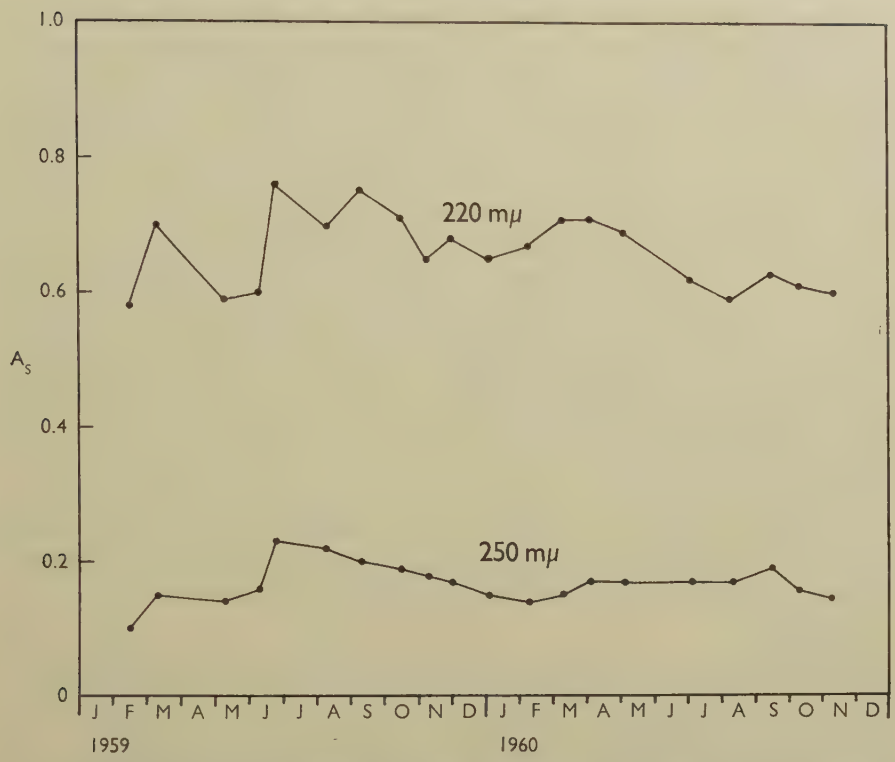

Fig. 3. Seasonal change in absorbancy at two wave-lengths, International Hydrographic Station E1 (50° 02′ N., 4° 22′ W.).

DISCUSSION

The results indicate that the increase in absorption at the blue end of the spectrum shown by Kalle to be characteristic of coastal waters, and confirmed by Joseph at 367 mμ and by Jerlov at 310 mμ, becomes even more marked below 300 mμ. The great difference between artificial and natural sea waters suggests that much of the ultra-violet absorption must be due to organic matter and it is reasonable to suppose that what is being measured is, at least in part, the absorbancy of Kalle's 'Gelbstoff' at short wave-lengths. This does not explain the change in character of the absorption spectra with increased depth in the north-east Atlantic and an interpretation is needed of the observations in the deep water samples, of lower absorbancy above 235 mμ and higher absorbancy below this wave-length. The former may be due, perhaps, to the differing origin and age of the deep water, and the latter effect may be ascribed to its higher nitrate concentration. Nitrate ion absorbs strongly in the short ultra-violet, with a maximum at about 203 mμ (Bastin, Weberling & Palilla, 1957). We find that its absorption at 220 mμ is considerable, and

calculate that nitrate concentrations of 15–20 μg atom NO_3-N/l. would account for the differences in absorbancy at this wave-length, between the deep and surface waters. Some observations in May 1961 gave values of 15–23 μg atom NO_3-N/l. between 500 and 2500 m at the station at 46° 30′ N., 8° 00′ W.

The nature of the organic matter in the sea is still mostly unknown. It has been reported that coloured material can be extracted with solvents (Shapiro, 1957) and if sea water is passed through active charcoal its ultra-violet absorption is decreased. Strongly coloured material can be extracted from the carbon with solvents (Wilson & Armstrong, 1952; Johnston, 1955). Fogg & Boalch (1958) have shown that coloured, and ultra-violet absorbent substances are excreted by a marine alga. The strong ultra-violet absorbancy of the Clyde sample may have been due in part to its high plant population, though it may also have been caused by land drainage. We understand (Dr H. Barnes, private communication) that it was taken after heavy local rainfall.

A further, though minor, part of the absorbancy is caused by that of volatile organic material. This can be measured separately on distillates from sea water (Armstrong & Boalch, 1960). This fraction, which we believe to be partly of algal origin may contribute up to about 5% of the absorption due to organic matter.

Measurements of ultra-violet absorption at these wave-lengths are easily made with commercial spectrophotometers and are quite as practicable at sea in the larger research ships as on shore. They may be useful in supplementing other optical measurements with specialized submersible apparatus, and should help in the physical classification of water masses, and possibly give some information on their chemical properties.

SUMMARY

Measurements of the ultra-violet absorption spectra between 200 and 400 mμ have been made with a spectrophotometer. At short wave-lengths natural sea water has double the absorbancy of artificial sea water. The difference in samples from shallow depths is ascribed to organic material, of which part may be the 'Gelbstoff' of Kalle. Regional variations have been found, coastal waters having higher U.V. absorptions. A small seasonal effect with an increase in absorbancy in summer in the English Channel has been seen. In deep Atlantic water increased absorption below 235 mμ may be due to its high nitrate concentration. At longer wave-lengths absorbancies were less than in surface waters. Measurements of U.V. absorption may supplement other physical methods of characterizing water masses.

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QUANTITATIVE DIFFERENCES IN THE FAUNA OF BOREAL MUD ASSOCIATIONS

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(With Text-figs. 1–3)

In studying the factors which may be regarded as limiting in benthic production, the selection of suitable locations for work is of major importance. Jones (1956) has discussed the requirements of the ideal location and stresses the importance of finding a large area where the deposit is stable and uniform, and where it is soft enough to allow adequate sampling with a grab. The Fladen ground in the centre of the northern North Sea satisfied these requirements and had the additional attraction that data were already available on the hydrography and basic production (Steele, 1956).

In searching for a ground to compare with Fladen, an area was required which differed in the biomass of the benthos, but which had at least some similarities to Fladen in environmental features. Because of the generally higher productivity of coastal waters an inshore ground seemed indicated, and certain Scottish west-coast sea lochs appeared to satisfy the requirements. After a general survey, Loch Nevis was selected for detailed study (Fig. 1).

The complete work involves regular surveys of the benthos made every 7 or 8 weeks throughout one year, coupled with simultaneous studies by Mr J. H. Steele of the basic plankton production and particulate organic carbon in the waters of the two areas. The general aim is to examine relationships between benthic production and the basic production of the plankton.

This initial paper describes the bottom fauna, and discusses the differences in the two areas on the basis of surveys made in autumn 1959.

I wish to thank the Director, Dr A. B. Stewart, and Dr H. R. M. Hardie, of the Macaulay Research Institute, Aberdeen, who kindly arranged for the mechanical analysis of the soils. The following have assisted with checking or identifying various groups: Dr R. H. Headley (Foraminifera), Dr W. G. Inglis (Nematoda), and Dr K. W. Ockelmann (Lamellibranchiata). Dr A. C. Stephen has kindly provided information and discussed with me his previous work in the northern North Sea. In particular I wish to acknowledge Mr J. H. Steele who has discussed his results with me and supplied the chlorophyll data of Fig. 3, p. 613, part of which is published elsewhere (Steele & Baird, 1961).

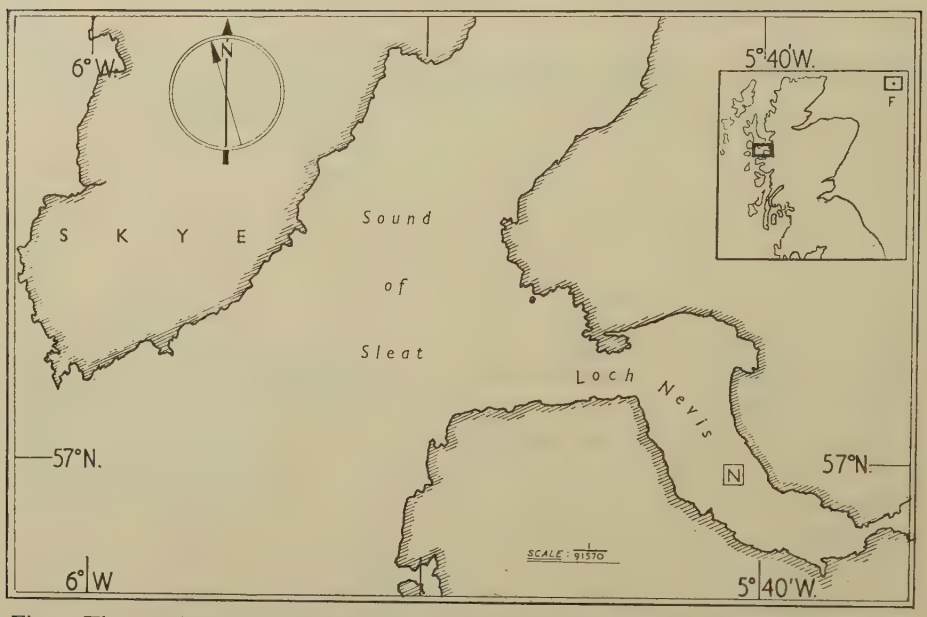

Fig. 1. The sampling position in Loch Nevis is shown by the square N. The inset shows the relative positions of the Fladen ground (F) and the Nevis area.

METHODS

Both grounds were examined by underwater photography and found to consist of uniform mud with little epifauna. In each area a number of samples (20 on Fladen, 15 in Nevis) were taken with a $\frac{1}{10}$ m^2 van Veen grab. The soft bottoms were ideal for this instrument which dug to depths of up to 15 cm and produced 9–16 l. of mud in each haul. The mud was washed first through a coarse sieve with round holes of 1·3 mm diameter, then through a fine sieve with square meshes of 0·5 mm side. From each haul the residues were preserved separately in 5 % formalin. The animals were later extracted, identified, counted, and the rough formalin weight determined after drying on blotting-paper. This weight includes shells and skeletal structures, as well as gut contents. Dry weights were not measured at this stage, because this will eventually be done on the basis of year-classes, and these cannot be defined accurately until material covering several seasons is available.

For meiobenthos collections a core sampler was used—a modification of the instrument described by Moore & Neill (1930). The cores were obtained in Perspex tubes of 4·3 cm inside diameter and dealt with as described on p. 607. The corer was also used to obtain samples from known depth in the mud for analysis of particle size and to provide unsieved samples for visual examination of the soil.

THE ENVIRONMENT

The first area, the Fladen ground, is in the northern North Sea roughly 100 miles north-east by east of Aberdeen; it is part of a flat muddy ground extending for several hundred square miles at a depth of about 80 fathoms. A preliminary survey of this whole area with camera and grab showed the fauna to be uniform. The station from which samples were taken covered only a few square miles centred on the position 58° 20′ N., 00° 30′ E., and the use of Decca made it possible to locate this station accurately.

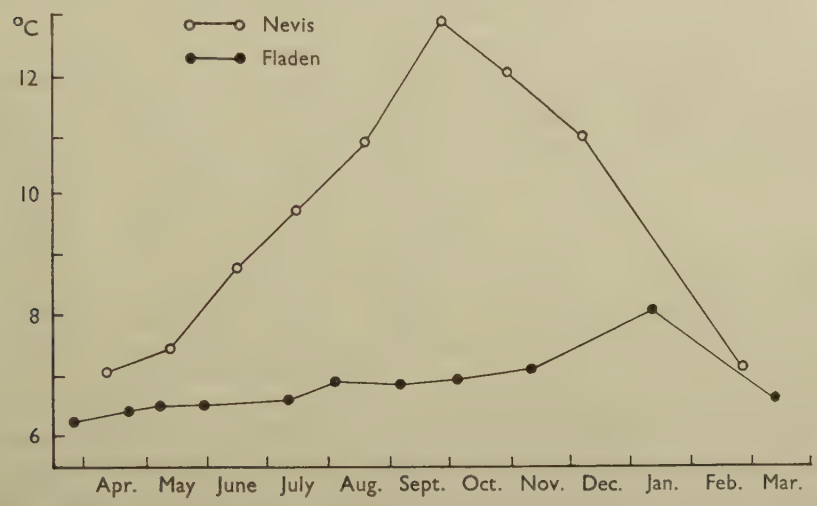

Fig. 2. Range of temperatures at the bottom in Nevis and Fladen throughout a complete year.

The annual range of bottom temperatures on Fladen is shown in Fig. 2. Salinities at the bottom vary throughout the year between 35·20 and 35·26‰.

The coastal area, Loch Nevis, is a sheltered western sea loch in Inverness-shire, opening into the Sound of Sleat. The main arm of the loch is between one and two miles wide, and the shores shelve steeply to over 80 fathoms at the deepest part. Across the entrance is a sill with its deepest channel at ten fathoms. The bottom samples were taken from a station roughly in the middle of the loch at a depth of 50–60 fathoms, a preliminary survey having shown this part to be representative of the loch as a whole. Accurate positioning was possible by the use of marks on the land.

Hydrographic observations in the Minch and in some western sea lochs have been published by Craig (1959), but details for Loch Nevis are lacking. The annual cycle of bottom temperatures at the sampling station during the year of the present survey is shown in Fig. 2. Salinities at the bottom ranged from 33·86 to 34·33‰.

A mechanical analysis for particle size was made of mud samples from the two areas, and the results are shown in Table 1, expressed as the percentage by weight of the sample passing through a 2 mm sieve. For each sample the difference between the sum of the fractions and 100 is due mainly to calcium carbonate and organic matter, both of which are destroyed in the preliminary treatment. In most samples a portion of between 14 and 20 % by weight of the original was retained in the 2 mm sieve. The analysis shows the Nevis deposit to be of finer particle size, the bulk being in the clay fraction, while on Fladen coarse silt predominates.

TABLE 1. SOIL GRADES

(See text for explanation.)

	Depth in mud in cm	Coarse sand 2·0–0·2 mm	Fine sand 0·2–0·05 mm	Coarse silt 0·05–0·02 mm	Silt 0·02–0·002 mm	Clay <0·002 mm
Fladen	0–1	1·4	10·9	39·5	18·6	17·4
	1–2	2·4	15·6	36·2	17·8	18·2
	2–3	0·7	19·9	34·5	19·2	18·2
	3–4	0·5	5·0	33·3	20·8	31·4
	4–5	0·5	15·1	38·4	18·1	20·1
Nevis	0–2	0·1	Nil	7·5	36·8	49·7
	2–4	0·2	2·4	13·7	24·9	51·0
	4–5	0·1	Nil	8·1	25·7	52·4
	5–6	0·2	Nil	10·4	29·9	52·1

While an analysis of this kind gives an accurate description of the basic particles present, it does not necessarily describe the actual conditions *in situ*. To study this, unsieved core samples from each 1 cm layer were examined. There was an obvious colour difference, the Fladen mud being greenish grey, and that from Nevis being distinctly brown, but there was no smell from the muds, and the minimum oxygen values from bottom water were between 4 and 5 ml./l. A more striking difference, even to the naked eye, was the presence in the Nevis mud of very large quantities of faecal pellets. The bulk of these could be identified as maldanid pellets, and many measured about 0·5 × 1·3 mm. Microscopic examination of the cores showed that the top 6–8 cm of mud in Nevis consisted largely of such pellets of this size or smaller, either intact or broken, together with large quantities of clay particles. Below this, faecal pellets were much less numerous, and many quartz particles and mica fragments were present, along with shells of Foraminifera.

On the top layers of Fladen mud, faecal pellets were comparatively few. A certain amount of fine silt was present and an appreciable quantity of quartz particles, some as big as 0·1–0·5 mm. But the striking feature of the Fladen mud was the presence of vast numbers of Foraminifera shells in all samples down to the deepest examined.

THE MACROBENTHOS

The numbers of animals per square metre are shown in Tables 2 and 3, the contents of coarse and fine sieves being listed separately. For the coarse-mesh sieve, the numbers are calculated from all hauls, 20 on Fladen and 15 in Nevis. The residues on these coarse sieves were of small volume, composed mainly of live animals with only a small proportion of extraneous matter which usually included valves of *Cyprina islandica* and *Chlamys septemradiata*. In the fine sieves, however, although the animals were numerous they were extremely small; the residues consisted of relatively large quantities of faecal pellets (in Nevis) or tests of Foraminifera (on Fladen) so that the extraction of the animals was most laborious. For this reason examinations of fine-mesh samples were confined to ten from each area and the animals were classified simply into main groups. Since this showed no major differences between samples, five were selected at random from each area for fuller identification, and the results were doubled to give numbers per square metre.

The Fladen Ground (Table 2)

This ground is included in the area, covering most of the deepest part of the northern North Sea, from which Stephen (1923) described the first Foraminifera community. The present results amplify his published data and can be compared directly with them, bearing in mind that he used a $\frac{1}{10}$ m^2 Petersen grab and a sieve with square meshes of 1·5 mm side. He gives a list of characteristic species of the community based on representative samples (two hauls at each of three stations), and also summarizes a larger number of hauls to show the abundance of the dominant organisms. Stephen bracketed the foraminifers *Saccammina* and *Psammosphaera* together, and there is still considerable uncertainty about the relationship of these two groups. In the present survey, tests retained in the 1·3 mm sieve were those of *Saccammina* only. Stephen (personal communication) states that his counts of these animals refer to intact tests, and his estimate of 1074 per m^2 is in close agreement with the corresponding value of 983 in the present work. However, a random sample of 1000 *Saccammina* tests were opened, and only 26·7 % were found to contain the animal. Of the rest 63·6 % were empty or contained only xanthosomes, while the remainder (9·7 %) contained unidentified enclosures of various types. The numbers of *Saccammina* are thus much less than suggested by a simple count of intact tests, but at 263 per m^2 this is still the dominant organism in the community. Another foraminifer found in large quantities by Stephen was *Astrorhiza arenaria*. In the new survey this was not common, but *Alveolophragmium crassimargo* tests were found in most hauls. As already noted, foraminifer tests dominated the residue in the fine-mesh sieves. No attempt has been made to find what proportion of these contained living animals, but estimates of dead tests give figures of over 10,000 per m^2 for

Saccammina, about 3000 for *Alveolophragmium* and over 15,000 for a species which Dr Headley has provisionally identified as *Rheophax scorpiurus* Montford. The latter inhabits a shell sometimes over 2·5 mm in length composed almost entirely of the calcareous tests of smaller Foraminifera.

Apart from Foraminifera, the most abundant group comprised Polychaeta, represented by 38 species and making up about 58% of the total weight.

TABLE 2. FLADEN STATION, OCTOBER 1959. NUMBERS OF INDIVIDUALS PER M²

	1·3 mm sieve	0·5 mm sieve
Saccammina spherica Sars	263	+
Astrorhiza arenaria Norman	4	—
Alveolophragmium crassimargo (Norman)	43	+
Pennatula phosphorea Linn.	2	—
Anthozoa	—	4
Thyasira equalis (Verrill & Bush)	71	14
T. croulinensis (Jeffreys)	12	16
T. ferruginea Winckworth	1	46
Cardium minimum Philippi	4	342
Cyprina islandica (Linn.) juv.	—	10
Abra nitida (Müller)	3	16
Cuspidaria obesa (Lovén)	2	—
Hiatella sp.	—	6
Natica sp. juv.	—	28
Prosobranchia	—	12
Cylichna sp. juv.	—	30
Opisthobranchia	1	86
Solenogastres	3	2
Dentalium sp. juv.	—	40
Macrocypris minna (Baird)	—	2
Macrocypria angusta G.O. Sars	—	14
Cythereis dunelmensis (Norman)	—	196
Leucon nasica (Kröyer)	3	12
L. acutirostris G.O. Sars	—	6
Eudorella emarginata (Kröyer)	1	—
Campylaspis costata G.O. Sars	3	—
Leptostylis villosa G.O. Sars	—	2
Apseudes spinosus (M. Sars)	2	—
Leptognathia filiformis (Lilljeborg)	—	14
Anarthrura simplex G. O. Sars	—	90
Pseudarachna hirsuta (G. O. Sars)	—	14
Ampelisca gibba G. O. Sars	1	—
Harpinia antennaria Meinert	2	10
H. crenulata Boeck	2	164
Arrhis phyllonyx (M. Sars)	1	—
Monoculodes packardi Boeck	2	—
Bathymedon longimanus (Boeck)	1	—
Eriopisa elongata Bruzelius	9	14
Amphipoda	—	8
Decapod larvae	4	—
Loxosoma sp.	+	—
Pholoë minuta Fabricius	—	4
Polynoidae	1	8
Paramphinome pulchella Sars	4	208
Phyllodoce sp.	1	—
Phyllodocidae	1	—
Ancistrosyllis groenlandica McIntosh	1	—
Ceratocephala borealis Wesenberg-Lund	1	2
Nereidae	1	2
Nephthys incisa Malmgren	14	62
Goniada maculata Oersted	1	2
Lumbrineris fragilis (Müller)	1	—
L. gracilis (Ehlers) / *L. impatiens* (Claparède)	11 / 18	10
Phylo norvegicus (Sars)	8	2
Paraonis gracilis oculata Hartman	84	132
Paraonidae	—	48
Skardaria fragmentata Wesenberg-Lund	1	2
Laonice sarsi Soderström	1	6
Nerine foliosa (Audouin & Milne Edwards)	1	—
Prinonospio cirrifera Wirén	2	10
Spiophanes kroyeri Grube	30	14
Tharyx multibranchis (Grube)	2	40
Tharyx sp.	25	30
Scalibregma inflatum Rathke	2	6
Ammotrypane aulogaster Rathke	7	8
Notomastus latericeus Sars	5	4
Capitellidae	4	6
Praxillella sp.	1	2
Rhodine loveni Malmgren	8	6
Myriochele heeri Malmgren	78	4
Owenia fusiformis delle Chiaje	1	—
Pectinaria sp. juv.	—	14
Anobothrus gracilis (Malmgren)	3	—
Samytha sexcirrata (Sars)	1	—
Ampharetidae	2	2
Terebellides stroemi Sars	1	—
Terebellidae	2	6
Sabellidae	1	—
Nemertini	+	+
Sipunculoidea	—	6
Turbellaria	—	4
Amphiura chiajei Forbes	2	4
Astropecten irregularis (Pennant)	4	134
Ophiura sp. juv.	—	16
Echinoidea juv.	3	8

Numerically, the thread-like *Paraonis gracilis* and *Myriochele heeri* dominated the collections, but *Spiophanes kroyeri*, *Tharyx* sp., *Lumbrineris impatiens* and *Nephthys incisa* were also important, and *Phylo norvegicus*, although not so abundant, made up a large part of the weight. Young specimens of *Paramphinome* were very numerous in the fine sieve, but adults were much less common. Apart from Polychaeta, only the lamellibranch *Thyasira* made a significant contribution to the biomass. Previous workers on Fladen have recorded *T. flexuosa*, but this species was not found in the present survey, when the common representative of the genus was *T. equalis*—a species which according to Ockelmann (1958) has in the past frequently been confused with *T. flexuosa*. Another notable feature of the lamellibranchs was the large number of small *Cardium minimum* about 0·8–1·0 mm in length. Among the Crustacea, the amphipod *Eriopisa elongata* occurred in more than half the hauls, and small specimens of *Harpinia crenulata* were very abundant.

Comparing the results from the two sieves (and leaving Foraminifera out of account) the fine mesh contained more than four times as many individuals as the coarse, but their weight was less than a quarter of that from the coarse. In general the fine-mesh animals were juvenile specimens, but there were some important exceptions to this—populations of tanaids (*Anarthrura simplex* and *Leptognathia filiformis*), isopods (*Pseudarachna hirsuta*) and small gastropods, groups which were scarcely or not at all represented in the coarse mesh. Also, certain groups found in the coarse mesh were represented in the fine by different species of smaller adult size, such as *Leucon acutirostris* and *Leptostylis villosa* among the Cumacea.

All the animals in the samples were small, less than 1 g formalin weight. The mean weight for all species was 6·42 g/m^2, and this weight was made up as follows:

Saccammina	Polychaeta	Nemertini	Mollusca	Crustacea	Echinodermata	Others
0·83	3·74	0·36	1·04	0·28	0·07	0·10

Loch Nevis (Table 3)

The dominant species numerically in Nevis was the foraminifer *Crithionina granum*, which was present in every haul. As described by Höglund (1947) these animals vary greatly in shape from spindle-like to oval or almost spherical. The greatest length of the largest specimen found in the present survey was 4·75 mm, but in spite of their abundance *Crithionina* made up only a small proportion of the total weight in the samples.

Apart from Foraminifera the main animals were polychaetes, of which 29 species were found. The maldanid *Rhodine loveni* was dominant, being present in every haul and accounting for over 68 % of the total polychaete weight. Another 15 % of this weight was made up by *Glycera rouxii* (due mainly to a few large specimens) while the much smaller *Lumbrineris impatiens* was important numerically. The brittle star *Amphiura chiajei* was found in every

haul and *Leucon nasica* (Cumacea) and *Philomedes macandrei* (Ostracoda) were present in most. Ten species of lamellibranch were present but none was particularly abundant, only *Thyasira flexuosa* being found in more than half the hauls.

In general the fine-mesh sieve contained only juvenile stages of the larger specimens retained by the coarse mesh. Most striking were the numbers of small *Philomedes* (about 1·0–1·4 mm long), *Prionospio malmgreni*, and the very abundant pieces of maldanids, which were often broken up into single segments, making accurate species identification and counts of individuals difficult. Mainly due to these, the fine-mesh counts were a little more than twice those of the coarse mesh, but the average weight was only about one-twentieth of the coarse mesh value.

Most of the animals sampled were less than 1 g formalin weight, the only

TABLE 3. LOCH NEVIS STATION, SEPTEMBER 1959. NUMBERS OF INDIVIDUALS PER M²

	1·3 mm sieve	0·5 mm sieve
Crithionina granum Goës	205	—
Perigonimus repens (Wright)	+	—
Nucula sulcata Bronn	9	4
Yoldiella tenuis (Philippi)	1	4
Thyasira flexuosa (Montagu)	7	2
T. croulinensis (Jeffreys)	2	18
T. ferruginea Winckworth	5	18
Cardium sp. juv.	—	4
Cyprina islandica juv.	—	2
Abra nitida (Müller)	5	40
Corbula gibba (Olivi)	5	—
Lamellibranchia unidentified	1	—
Opisthobranchia	—	16
Solenogastres	11	40
Dentalium juv.	—	8
Philomedes macandrei (Baird)	35	630
Cythereis jonesi (Baird)	—	4
Leucon nasica (Kröyer)	25	4
Eudorella emarginata (Kröyer)	3	6
E. truncatula (Bate)	—	2
Leptostylis villosa G. O. Sars	—	2
Lysianassidae	1	—
Leucothoë lilljeborgi Boeck	1	2
Nicippe tumida Bruzelius	1	—
Perioculodes longimanus (Bate & Westwood)	—	2
Westwoodilla acutifrons (G. O. Sars)	1	—
Eriopisa elongata (Bruzelius)	3	12
Desmosoma filipes Hult	—	2
Leptognathia brevimana (Lilljeborg)	—	2
Processa canaliculata Leach	1	—
Calocaris macandreae Bell	3	—
Other Crustacea juv.	—	2
Pholoë minuta Fabricius	—	8
Polynoidae	1	—
Ophiodromus flexuosus (delle Chiaje)	1	12
Hesionidae	1	—
Ancistrosyllis groenlandica McIntosh	5	4
Nephthys hystricis McIntosh	7	6
Glycera alba (Müller)	1	—
G. rouxii Audouin and Milne Edwards	12	2
Lumbrineris impatiens (Claparède)	21	8
Errantia unidentified	1	14
Skardaria fragmentata Wesenberg-Lund	—	2
Paraonis gracilis Tauber	—	14
Paraonidae	4	—
Prionospio cirrifera Wirén	5	8
P. malmgreni Claparède	8	108
P. steenstrupi Malmgren	2	—
Prionospio sp.	3	12
Spiophanes kroyeri Grube	7	—
Chaetozone setosa Malmgren	2	8
Tharyx marioni (Saint-Joseph)	5	4
Diplocirrus glaucus (Malmgren)	5	8
Lipobranchius jeffreysii McIntosh	1	—
Dasybranchus caducus (Grube)	2	—
Praxillella gracilis (Sars)	7	+
Praxillella sp.	2	
Rhodine loveni Malmgren	71	
Amphitrite cirrata O. F. Müller	1	—
Pista cristata (Müller)	1	—
Sedentaria unidentified	1	6
Nemertini	1	+
Amphiura chiajei Forbes	32	+
A. filiformis (O. F. Müller)	1	
Loxosoma sp.	+	—
Triticella koreni Sars	+	—

exceptions being one large *Glycera rouxii* and three specimens of *Calocaris*, each between 1 and 2 g weight. The average formalin weight for all species was 33·10 g/m^2, and this was made up as follows:

Crithionina	Polychaeta	Nemertini	Mollusca	Crustacea	Echinodermata
1·77	13·68	0·03	2·17	4·09	11·36

THE MEIOBENTHOS

In considering elements of the fauna smaller than those usually sampled by grabs, it is generally accepted that their separation from the substratum and subsequent identification is best carried out on living material. The sampling conditions thus imposed and the laborious techniques usually involved make it difficult to combine such work with studies of the larger animals. Mare (1942) dealt with the total fauna of a marine mud, and divided it into macro-, meio-, and micro-benthos, but there is a tendency for workers to select a particular size-group of the fauna, and to concentrate on it exclusively. In recent years the macrobenthos has been quite extensively studied and the smaller groups comparatively neglected.

In the present work it was felt that an adequate comparison of the two areas must include estimates at least of the meiobenthos. Accepting that this had to be included as only one part of an extensive series of observations at sea, and could not therefore be dealt with as fully as would otherwise be desired, the following simplified routine was developed.

Immediately on collection the top 8 cm of each core was cut into 1 cm parts, and each part washed through a British standard sieve of 0·124 mm mesh. Any large individuals, which would have been retained by the 0·5 mm sieve, were removed. The residues were stained in an aqueous solution of rose bengal and finally preserved in 70% alcohol. This procedure is not ideal or complete in itself, since occasional live collections must be examined if total species identification of all the less robust groups is required. However, the procedure does give numerical estimates of the various groups, and is sufficiently straightforward to enable it to be done in conjunction with work on the macrofauna. The stain, which was originally used to distinguish between living and dead Foraminifera, is taken up also by copepods, nematodes, and other small animals, greatly assisting their extraction from the residue.

A full account of the meiobenthos, based on samples taken throughout the year, will be published elsewhere. In the present paper, details are restricted to four cores taken at the same time as the grab samples—two from Fladen and two from Nevis. From these, the average numbers of animals per $\frac{1}{100}$ m^2 surface area are shown in Table 4.

Most of the fauna was confined to the top 2 cm of the mud, and below 4 cm only a few nematodes and an occasional polychaete or lamellibranch were found.

The fauna falls into two distinct parts. First there are those elements which are juvenile stages of larger animals—the lamellibranchs, gastropods, and most

of the polychaetes. These are temporary members of the meiobenthos, and considerable seasonal fluctuation in their numbers may be expected. The autumn counts for these from Nevis and Fladen were 118 and 281 individuals per $\frac{1}{100}$ m^2, representing about $\frac{1}{15}$ and $\frac{1}{5}$ of the total meiobenthos respectively.

But the greater part of the fauna from the cores may be regarded as permanent meiobenthos, since it consisted of species which in the adult stage were still too small to be retained by sieves normally used for grab samples. The permanent meiobenthos in Nevis was rather greater than on Fladen (1543 individuals compared with 976 individuals per $\frac{1}{100}$ m^2). The main group was Nematoda—specimens varying in length from 0·5 mm to just over 2·0 mm. They were found as far down in the mud as the sixth centimetre layer,

TABLE 4. MEIOBENTHOS. AVERAGE NUMBERS OF ANIMALS PER ONE-HUNDREDTH OF A SQUARE METRE FROM TWO CORES IN EACH AREA

	Fladen	Nevis
Foraminifera	122	290
Nematoda	618	861
Kinorhyncha	3	10
Copepoda	223	284
Ostracoda	10	98
Polychaeta	78	105
Lamellibranchiata	149	10
Gastropoda	51	0
Other Groups	3	3

but the bulk of the population was concentrated in the second centimetre. More than half the individuals belonged to the families Oncholamidae and Comestomidae (with species *Dorylaimopsis punctatus* Ditl. and *Sabatieria cupida* Bressl. & Sch. St. prominent).

The copepods were all harpacticoids between 0·2 and 1·0 mm long, and were almost entirely confined to the surface centimetre of the mud.

Foraminifer shells (particularly abundant on Fladen) were present at every depth in the mud, but specimens which took up the stain were confined to the surface centimetre. In making counts, only these stained specimens were regarded as having been alive at the time of collection, but a possible source of error should be noted here—that some of the tests may have been inhabited by foreign organisms. In Nevis the main species of Foraminifera were *Bulimina marginata* d'Orb. with a few *Cancris auriculus* (Fichtel & Moll) and *Quinqueloculina seminulum* (Linn.). Among the main living species on Fladen, *Bulimina marginata* was again common, along with *Uvigerina peregrina* Cushman and *Nonion umbilicatula* (Walker & Jacob). The Fladen deposit contained very great numbers of empty tests, in particular of *N. umbilicatula* and the very similar *N. pompiloides* (Fichtel & Moll).

Total numbers of meiobenthos were $1{\cdot}26 \times 10^5$ individuals per m^2 on Fladen and $1{\cdot}66 \times 10^5$ per m^2 in Nevis. Accurate direct weighing of small

organisms is difficult because of evaporation, but a rough approximation was desired so that comparison with macrobenthos could be made. By weighing large numbers of various groups on a cover-glass, estimates of average individual weights were obtained for Foraminifera (0·010 mg), Polychaeta (0·120 mg) and Ostracoda and Mollusca (0·022 mg). The best averages arrived at by Mare (1942) were used for Nematoda (0·003 mg) and Copepoda (0·005 mg). This gives estimates for total meiobenthos of 1·820 g/m^2 for Fladen and 2·198 g/m^2 for Nevis. These values refer to individuals which can pass the 0·5 mm but are retained on the 0·124 mm sieve. A large proportion of the weight is made up by Polychaeta and Mollusca (Fladen 75·6 % and Nevis 58·3 %).

NOTES ON CERTAIN SPECIES

HYDROIDA. *Perigonimus repens* was present on all specimens of *Nucula sulcata* from Loch Nevis.

POLYZOA s.str. *Triticella koreni*. All the *Calocaris macandreae* from Loch Nevis carried this species abundantly on the body and appendages. It was also found on *Calocaris* collected from Fladen in later surveys.

KAMPTOZOA. *Loxosoma* sp. Many specimens were found associated with the maldanid *Rhodine loveni* from both Fladen and Nevis. They were attached to the surface of the worm and also to the inside of the tube.

Many *Nephthys* from Fladen and Nevis carried what was probably a species of *Loxosoma* but it has not yet been possible to examine these alive.

POLYCHAETA. Records of special interest have been discussed in a separate paper (McIntyre, 1961). The only addition to be made here is two further specimens of *Skarderia fragmentata*, one from Fladen and one from Loch Nevis. Both were short anterior fragments. The Nevis record extends the known distribution of this species, which has been recorded previously only from south-east Iceland (1 specimen) and Fladen (1 specimen).

MOLLUSCA. *Thyasira equalis* (see Verrill & Bush, 1898, pl. 91, figs. 5–6). The identity of the Fladen specimens has been confirmed by Ockelmann. He states (personal communication), after examining type material of this species, that it is identical with his '*Thyasira* spec. A' (Ockelmann, 1958, p. 100) and that his '*Thyasira equalis*' is probably an undescribed species.

AMPHIPODA. *Westwoodilla caecula* (Bate). The single specimen from Loch Nevis had a cephalon as described for *W. acutifrons* (Sars), but Enequist (1950) considers that the latter should be united with *W. caecula* which shows great variability.

DISCUSSION

Comparison of the faunas

The Fladen and Nevis faunas had many similarities. Both were characterized by aranaceous Foraminifera, dominated by Polychaeta, and had close

parallels in species composition. The following species were common to both grounds:

Pholoë minuta
**Panthalis oerstedi* Kingberg
Ancistrosyllis groenlandica
Lumbrineris impatiens
Skardaria fragmentata
Paraonis gracilis
Spiophanes kroyeri
Prionospio cirrifera
Rhodine loveni
**Diplocirrus glaucus*
**Terebellides stroemi*
Amphiura chiajei
**Nucula tenuis* (Montagu)
Cardium minimum
Thyasira ferruginea
T. croulinensis
Cyprina islandica
Abra nitida
Leucon nasica
Eudorella emarginata
Leptostyllis villosa
Eriopisa elongata
**Calocaris macandreae*

Those marked with an asterisk were recorded on one or both grounds from surveys more recent than those so far described in this paper. As well as the above, the following genera were represented by closely related species on each ground: *Nephthys*, *Praxillella*, *Tharyx*, *Cythereis* and *Leptognathia*.

The Fladen Foraminifera community has been known for some time and has been reviewed in relation to other similar communities by Thorson (1957). In the classification of Jones (1950), based on physical conditions, it is referred to as a boreal deep mud association, distinguished by depth greater than 70 m, temperature about 3–7° C and salinity 34–35·5‰.

Comprehensive benthos studies have not previously been made in Scottish north-west coast lochs, so one may consider the relation of the Nevis community described here to other known associations. It is obviously parallel to the arctic Foraminifera communities described by Spärck (1933) and Thorson (1934) from north-east Greenland fjords. These are characterized by *Rhabdamina cornuta* and *Miliolina bucculenta* and have weights occasionally over 50 g/m^2. However, such high weights were usually caused by a few large animals (e.g. echinoderms and ascidians) and when these and Foraminifera are left out weights are seldom more than 5–6 g/m^2, often much less. Considering boreal communities, the Nevis fauna has much in common with the *Rhodine* facies of the *Brissopsis-chiajei* association described by Molander (1928) from the Gullmar fjord. But the depth of the Nevis community is greater than that specified by Molander, and the salinity is higher and much less variable. The recorded community which most resembles Nevis is that of Fladen. They have much the same depth, bottom type, and salinity, but there is an important difference in the temperature (Fig. 2). This suggests that the Nevis fauna is an 'offshore' rather than a 'deep' mud community in the sense used by Jones.

The differences between the two faunas were mainly quantitative. In the

meiobenthos, although Fladen had large numbers of recently settled juvenile molluscs, Nevis had the greater overall population, and considering only the permanent representatives of the meiobenthos, Nevis had half as many again as Fladen.

The macrobenthos weights were 6·43 g/m^2 for Fladen and 33·10 g/m^2 for Nevis. The higher weights in Nevis were not restricted to any one section of the fauna, but were found in all the main animal groups. The abundant population of *Amphiura chiajei* in Nevis contrasted with the poor one on Fladen. Comparatively large animals like *Calocaris* and *Processa* made up about 95 % of the Nevis crustacea weight, but even when these are excluded it was twice as great as that for Fladen. Considering the lamellibranchs, Fladen supported a good population of *Thyasira equalis* (71 individuals/m^2), but in spite of this the total mollusc weight for Nevis was 2½ times that of Fladen. Among the errant polychaetes *Nephthys* and *Lumbrineris* were more abundant and heavier on Fladen, but the Nevis total was greater, due mainly to *Glycera rouxii*. As for the sedentary polychaetes, *Spiophanes kroyeri* and *Tharyx* sp. were well represented on Fladen, but apart from these the numbers were largely made up of small, thread-like forms—*Paraonis* and *Myriochele*, which contributed little to the weight. The dominant in Nevis, on the other hand, was *Rhodine loveni* which made up over 68 % of the total polychaete weight in the coarse sieve, and helped to account for the Nevis polychaete weight exceeding that of Fladen by a factor of almost four.

Causes of the difference

The two grounds, then, maintain faunas which are very similar qualitatively but which differ overall in biomass by a factor of more than five. When examination of seasonal samples is completed, giving data on reproduction and growth rates, and estimates of productivity rather than standing crops, it should be possible to discuss the reasons for the difference with more understanding. At this point it is worth simply noting two possible factors—predation and hydrographic conditions.

Predation by fish

Information is not available on the abundance of fish in Loch Nevis, but from what is known of other west-coast lochs, large permanent fish populations would not be expected. The Fladen area, on the other hand, is well documented. It is fished by commercial trawlers from September to March, and seine-net vessels work there more or less throughout the year. Herring are landed in quantity but may be ignored here, since they do not feed from the bottom. The main demersal species in the commercial landings from Fladen are haddock and whiting. Their food has been studied by Jones (1954) who concluded that 'the whiting is an active predator, feeding primarily on fish

and more active crustaceans, chiefly Decapoda Natantia and amphipods, while the haddock is more exclusively a bottom feeder'. Ritchie (1937) studied the food of the haddock in considerable detail. He states that on northern North Sea grounds east of the Greenwich meridian, haddock feed largely on annelids, but he distinguishes the Fladen as an area where in some parts of the year they concentrate almost exclusively on the lamellibranch *Cardium minimum*. He gives measurements of this species from haddock stomachs varying between 1 and 2·5 mm (mostly 1·5–2·0 mm) and suggests that Stephen may have failed to record this in his survey because of the large mesh of his sieve. Ritchie also states that he never found *Thyasira* in the haddock stomachs he examined. It could be argued on the basis of Ritchie's data that the large standing crop of *Thyasira* and the small numbers of adult *Cardium* in the present survey may be explained by haddock predation. On the other hand, the standing crop of such polychaetes as *Nephthys* was comparatively large, although Ritchie found these abundantly in haddock stomachs. Until further data are available (such as estimates of benthos brood survival on Fladen and Nevis) it is difficult to decide finally how important predation may be in accounting for the fauna differences.

Hydrographic factors

In seeking to explain biomass differences of benthos on two grounds in the Irish Sea, Jones (1956) dismissed as possible factors the organic carbon and the total phosphorus contents of the deposits, but noted differences of phosphorus levels in the surface waters. He therefore tentatively postulated corresponding differences in plankton production which might account for the higher biomass of the benthos-rich areas, and suggested that total phosphorus in the overlying water might be limiting to benthos production.

In Loch Nevis, measurements of phosphorus at several depths between surface and bottom during the winter maxima averaged 0·66 μg at./l. and the corresponding value for Fladen was 0·68 μg at./l. This shows little difference between the two areas. However, the graphs of temperatures (Fig. 2) indicate hydrographic differences which should be considered.

The situation on Fladen has been studied by Steele (1956). In winter, relatively warm Atlantic water, flowing round the north of Scotland, enters the northern North Sea and mixes with water already there. On the Fladen ground at this time there is complete vertical mixing of the water column, and the highest annual bottom temperatures of over 8° C are recorded. Cooling takes place until the spring, when the surface temperatures begin to rise, and a thermocline is set up. The thermocline depth varies from 30 m to about 70 m later in the season, and large scale hydrographic changes are confined to water above the thermocline. Below this lies the cold stable lower water where, even though surface temperatures in August reach about 14° C, the

bottom temperature scarcely exceeds its late winter level of 6–7° C until the thermocline breaks down late in the year. Slightly higher temperatures—the annual maxima—are then produced. This limited range of bottom temperature is characteristic of 'constant-boreal water' as described by Hult (1941).

In Loch Nevis, bottom temperatures for the first 6 months of the year are not far above those of Fladen (Fig. 2), but the Nevis thermocline set up in spring is weaker than that on Fladen. The increase in bottom temperature during the summer shows that an exchange with surface waters is occurring,

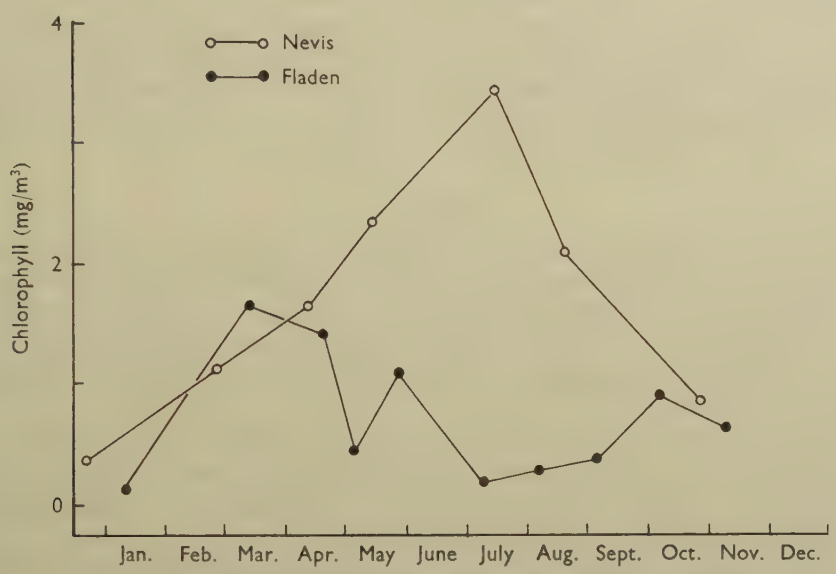

Fig. 3. Chlorophyll (mg/m³) in Fladen and Nevis at 10 m depth.

and the thermocline in Nevis breaks down completely much earlier—about September. At this time, when the warm surface waters are mixed downwards, the highest bottom temperatures of about 13° C are recorded. From then until about May, when the re-establishment of the thermocline begins, there is complete mixing of the water column. The comparatively wide range of bottom temperatures produced by the above hydrographic condition is the essential characteristic of Hult's 'varying-boreal water'.

An important result of the poor vertical mixing on Fladen from early spring to late autumn is that the supply of nutrients to the euphotic zone is restricted during the season when primary production could occur, so that this production is kept to a low level. In Nevis the greater mixing can be expected to produce higher production. This is shown by a comparison of chlorophyll data from the upper layers of the two areas (Fig. 3), which indicates that primary production in Nevis is probably several times greater than on Fladen. One might

therefore expect a higher rate of deposition of organic material from upper waters on to the bottom in Loch Nevis, and that this could support greater populations of benthic animals.

If, in general, poor primary production may be expected from upper layers overlying 'constant-boreal water' then bottom communities in such areas may usually be poorer than those in 'varying-boreal water', just as Fladen is poorer than Nevis. It is difficult to check this from the literature on other areas because of the different methods used in expressing weights, but Molander's data from the Gullmar fjord support it. The average weights in his *Brissopsis–chiajei* association are more than double those of his *Melinna–tenuis* and *Terebellides–flexuosa* associations. It thus appears that the Jones's division into offshore and deep communities may have general quantitative as well as qualitative implications.

SUMMARY

The fauna of a muddy area in the northern North Sea (the Fladen ground) is compared with that of a similar area in a Scottish west-coast sea loch (Loch Nevis). These areas were selected because certain similarities in environmental features and in fauna, coupled with their marked difference in biomass, make them particularly suitable for studies of the factors affecting benthic production.

For macrobenthos samples (from a grab) a coarse sieve with round holes of 1·3 mm diameter and a fine sieve with meshes of 0·5 mm side were used. Meiobenthos samples (from a corer) were sieved through a 0·124 mm mesh and the total residue stained in rose bengal.

The Fladen ground, depth 140 m, had annual bottom temperatures between 6·31 and 8·22° C and salinities between 35·20 and 35·26‰. The deposit was mainly of coarse silt with abundant tests of small Foraminifera. The fauna is redescribed. The characteristic species was the foraminifer *Saccammina sphaerica*. By separating empty tests from living specimens it is shown that this species was not so abundant as had been thought. The main lamellibranch was *Thyasira equalis*, not *T. flexuosa* as recorded in previous work. Other important species were the polychaetes *Paraonis gracilis*, *Myriochele heeri*, *Spiophanes kroyeri*, *Tharyx* sp., *Lumbrineris impatiens*, and *Nephthys incisa*. The standing crop of macrobenthos was 6·42 g/m^2 wet weight.

At the Loch Nevis station, depth 90–110 m, bottom temperatures ranged between 7·04° and 12·90° C annually, and salinities between 33·86 and 34·33‰. The bottom deposit was mainly of clay, but very large numbers of faecal pellets were present. The fauna—a Foraminifera community characterized by *Crithionina granum*, is described. The main species were *Rhodine loveni* and *Lumbrineris impatiens* (Polychaeta), *Philomedes macandrei* (Ostra-

coda), *Leucon nasica* (Cumacea) and *Amphiura chiajei* (Echinodermata). The standing crop of macrobenthos was 33·10 g/m^2 wet weight.

The meiobenthos in both areas consisted of a permanent population of nematodes, harpacticoids, foraminifers, and ostracods. Young stages of molluscs and polychaetes were also present. There were $1{\cdot}26 \times 10^5$ individuals of the meiobenthos per m^2 on Fladen and $1{\cdot}66 \times 10^5$ per m^2 in Nevis. The corresponding weights were 1·820 and 2·198 g/m^2 respectively.

The two faunas are compared in detail and their relations with other known communities considered.

Causes of the biomass difference are discussed. Predation by fish is noted as a possible cause but hydrographic differences also may be important. The greater exchange between bottom and surface waters during the summer in Nevis leads to quicker returns of nutrients to the surface and hence to greater primary production which could favour a richer bottom fauna.

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VITAMIN A COMPOUNDS IN THE OVARIES OF THE COD, *GADUS MORHUA* L., FROM THE ARCTIC

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(Plates I and II and Text-figs. 1–4)

The occurrence of vitamin A_1 aldehyde in the ripe eggs of the herring (*Clupea harengus* L.), the cod (*Gadus morhua* L., see Cohen, 1959) and several other marine teleosts has recently been reported (Plack, Kon & Thompson, 1959). A study of vitamin A aldehyde and related substances in cod ovaries at different stages of maturation has now been made, which shows that the aldehyde is only present in ovaries containing ripening eggs.

MATERIALS AND METHODS

The cod (*Gadus morhua* L.) used for this study were from the Arcto-Norwegian stock which spawns in winter off the northern coast of Norway, mainly around the Lofoten Islands, and is distributed throughout the Barents Sea to Spitzbergen, Hope Island and Novaya Zemyla. The fish were trawl-caught by the Ministry of Agriculture, Fisheries and Food research vessel 'Ernest Holt'. Since the cod of this stock make extensive seasonal migrations covering several hundred miles it was necessary to take samples at different positions along the migratory routes during the year. During the summer months the cod are found in the shallow-water feeding grounds in the north and east of the Barents Sea. In late September, as the gonads begin to ripen, the fish leave the feeding areas to migrate distances of up to 800 miles to the spawning grounds. The main spawning season is from mid-February to the end of March. After spawning the spent cod return again to the Barents Sea feeding areas. These movements have been described in detail by Trout (1957) and are summarized in Text-fig. 1, in which the positions where the samples were obtained are also shown. The dates at which the samples were taken are given in Table 1.

The aim was to analyse for vitamin A compounds about ten pairs of ovaries

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Text-fig. 1. Chart of Barents Sea showing seasonal movements of the adult cod population. Numerals show the position at which each sample of ovaries was taken (see Table 1). —, Spawning migration; – – –, return of spent fish; hatched region, spawning area.

TABLE 1. DATA OF THE ARCTIC SAMPLES OF COD

Sample no.	Date of catch	Mean length of cod (cm)	No. of pairs of ovaries
1	18 October 1958	80	5
2	30 November 1958	83	8
3	3 February 1959	82	4
4	25 April 1959	83	8
5	12 June 1959	—*	9
6	27–28 August 1959	86	11
7	18 October 1959	87†	10
8	27 November 1959	90	11
9	17–19 February 1960	91	19

* Length measurements not recorded, but ovaries taken from fish between 80 and 90 cm.
† Mean value for five fish, the other five not measured.

from each sampling, but the weather at sea sometimes limited the number of suitable fish caught, so that this aim was not always achieved. In February 1960 the ovaries from a larger number of fish were collected, since from previous results they were expected to contain vitamin A aldehyde.

HISTOLOGICAL STUDIES

For histological examination ovaries were taken from freshly caught adult cod and fixed in Bouin's fluid. In fish at the beginning of maturation and in spent fish the complete right ovary was dissected out for fixation, but in ripening females, in which the ovaries weighed from 200 to 1000 g, a transverse section about 2 in. thick was cut out of the right ovary, and fixed. When the fish were running ripe, larger pieces were removed, and subsequently cut down after a preliminary fixation of 2 or 3 h.

The ovaries were embedded in wax, routinely sectioned at 10 μ, and stained with Heidenhain's haematoxylin and eosin, and with Masson's trichrome stain. The diameter of the eggs was measured under a microscope. Usually 500 measurements were made on each slide and the mean egg diameters calculated. Details of the cytological changes occurring in the ovary were recorded at the same time.

BIOCHEMICAL STUDIES

Collection and storage of ovaries

Freshly caught fish were either stored whole in the ship's deep-freeze at $-25°$ C or the ovaries were removed and stored in Polythene bags and the remainder of the fish discarded after its length had been measured. It was originally intended that whole fish should be sent to Shinfield, but the low storage capacity of the ship's deep-freeze, the increase in the number of samples and the general inconvenience of the bulk of the whole fish, led to ovaries being dissected out on the boat and stored as such. This method unfortunately meant that, at times, some fish were not measured. The samples were taken back to Lowestoft in insulated canisters and stored in deep-freeze until they were taken by insulated lorry to Wokingham and by van to Shinfield. There, samples of ovaries were placed in deep-freeze, but whole fish were thawed, measured and the ovaries removed and stored in Polythene bags in deep-freeze. The total storage time varied between 4 and 24 weeks for different batches of material.

Chemicals

Light petroleum, b.p. 40–60° C, n-hexane, and chloroform were used as supplied by May and Baker Ltd. Aluminium oxide for chromatographic adsorption analysis (B.D.H. Alumina) was obtained from British Drug Houses Ltd, and activated alumina, Type O (P.S. Alumina) from Peter Spence and Sons Ltd, Widnes. Other chemicals were of A.R. quality.

Preparation of sample

The ovaries were thawed overnight in a refrigerator at 4° C, and then weighed. Of the five pairs of ovaries from fish caught in October 1958, four were combined and analysed as one sample, and of the eight pairs from fish caught in November 1958, six were combined and analysed as one. Two small pairs of ovaries from fish caught in June 1959 were also combined, but otherwise the ovaries from one fish were analysed as one sample. The smaller ovaries were cut into pieces with scissors and mixed with a spatula. With the larger ovaries of February 1960, the tissue was homogenized in a Waring Blendor. From each sample a portion of about 5 g was weighed and dried at 105° C for the determination of dry matter. Another sample of 30 g (or the whole of the remainder, if less than 30 g) was weighed and homogenized in 10 g lots, each with 7·5 ml. water, in a Potter–Elvehjem homogenizer with a nylon pestle.

Extraction of lipids

The three homogenates were transferred to a Waring Blendor, together with one washing of the homogenizer with 7·5 ml. water. The sample was blended with 200 ml. light petroleum in an atmosphere of nitrogen. Absolute ethanol, 60 ml., was then added with the Blendor running and the tissue was extracted for about 2 min. The upper light-petroleum layer was poured off into a brown one-litre conical flask and the residue extracted twice more with 200 ml. lots of light petroleum. The combined extracts were allowed to settle for 2 h or more and the clear supernatant liquid was evaporated in a tared flask on a steam-bath, the last portions of solvent being removed under reduced pressure with little or no heating. After weighing, the extracted lipids were dissolved in n-hexane and the volume made up to 12 ml.

Determination of vitamin A aldehyde

In the routine method a 4 ml. sample of the extract was put on to a column of water-weakened P.S. Alumina, the chromatogram developed by gradient elution and the aldehyde fractions combined as described by Plack *et al.* (1959). Vitamin A aldehyde was determined by the intensity at 664 mμ of the blue colour in the Carr–Price test (see Plack *et al.*, 1959).

In the later stages of the experiment now reported a method became available for the determination of vitamin A_1 and A_2 aldehydes in the presence of each other. The method has been described by Plack & Kon (1961), who applied it to the aldehyde fractions from the gradient elution, since the two aldehydes are eluted at almost the same rate.

Determination of vitamin A esters and alcohol

Vitamin A esters together with vitamin A aldehyde, and vitamin A alcohol were separated by chromatography of 4 ml. samples of the extracts on

ethanol-weakened B.D.H. alumina, as described by Thompson, Ganguly & Kon (1949), with the modification of Kon, McGillivray & Thompson (1955). The two fractions were saponified and extracted as described by Plack (1956), except that the aqueous KOH solution was 60% (w/w). Saponification destroys the vitamin A aldehyde and converts vitamin A esters into the alcohol. Each fraction was subjected to a further chromatography on ethanol-weakened B.D.H. alumina, and the residues from the eluates obtained with 8% ethanol in n-hexane were dissolved in chloroform for the Carr–Price test (see Plack *et al.*, 1959). A test was made for vitamin A_2 esters and alcohol by Carr–Price readings at 693 mμ.

Estimation of carotenoids

A rough estimate of the total carotenoids present in the ovarian tissue was made by diluting a portion of the extract with an appropriate volume of n-hexane and determining the absorption curve in the range 380–540 mμ with an Optica CF4 double-beam recording spectrophotometer. A value of $E^{1\%}_{1\,cm} = 2300$ was used to calculate the quantity of carotenoids present from the maximum extinction in the region 470–480 mμ.

RESULTS

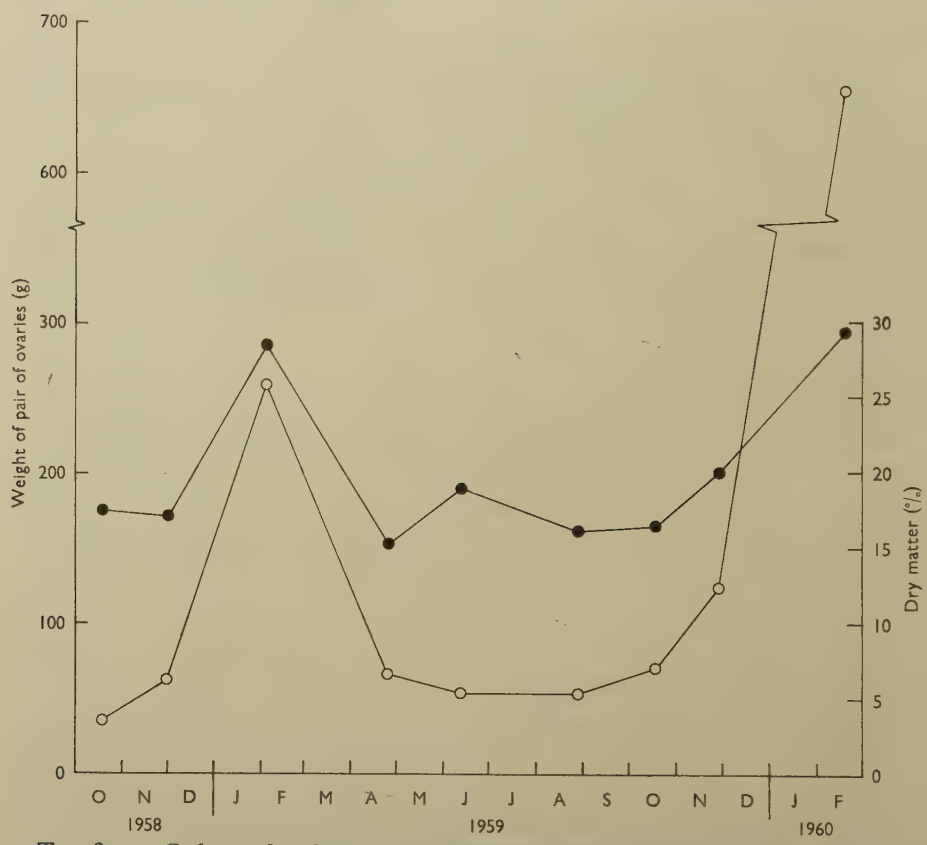
Size of samples and length of fish

In Table 1 dates of catch are shown together with the number of pairs of ovaries analysed. Fish of roughly the same size were selected for each sample, and from October 1958 to April 1959 their length was reasonably constant at about 80 cm; these cod would typically be 7–8 years old. Since the growth rate of Arctic cod varies seasonally, growth occurring mainly during the summer feeding period, the later samples were taken from larger fish (Table 1) so that during the second maturation cycle the length was about 90 cm (the size to which 80 cm cod would be expected to grow during one year in the Barents Sea), and the age of the fish would be 8–9 years.

Weight and dry-matter content of the ovaries

With the series of ovaries used for the biochemical work, the mean weight of a pair of ovaries was highest in February (Text-fig. 2), though in February 1960 this maximum was over twice that in February 1959. From April to August the ovarian weight showed little change, but in October, as the ovaries began to mature, their weight increased.

The dry-matter content of the ovaries increased with their maturation, but though the ovaries had begun to increase in weight in October, their dry-matter content did not increase until a later stage of the ripening process (Text-fig. 2).



Text-fig. 2. *Gadus morhua* from the Arctic. Mean values for the weight (○—○) and percentage of dry matter (●—●) of a pair of ovaries during the maturation cycle.

Histological changes in maturing ovaries

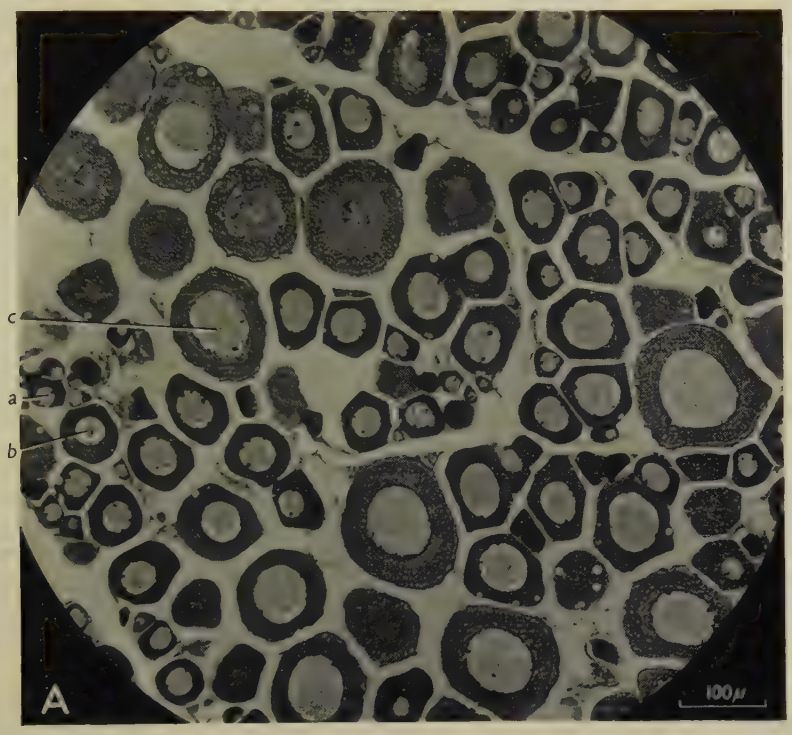
Throughout the summer months of June to September, when cod were feeding, the ovaries were inactive. Three types of egg cell could be distinguished in microscope sections (Pl. I A), the smallest being the oogonia which occurred in clusters throughout the ovary. There were also numerous small primary oocytes, which were round, deeply staining cells of 25–65 μ diameter, with a large nucleus in which three to six prominent nucleoli were apparent.

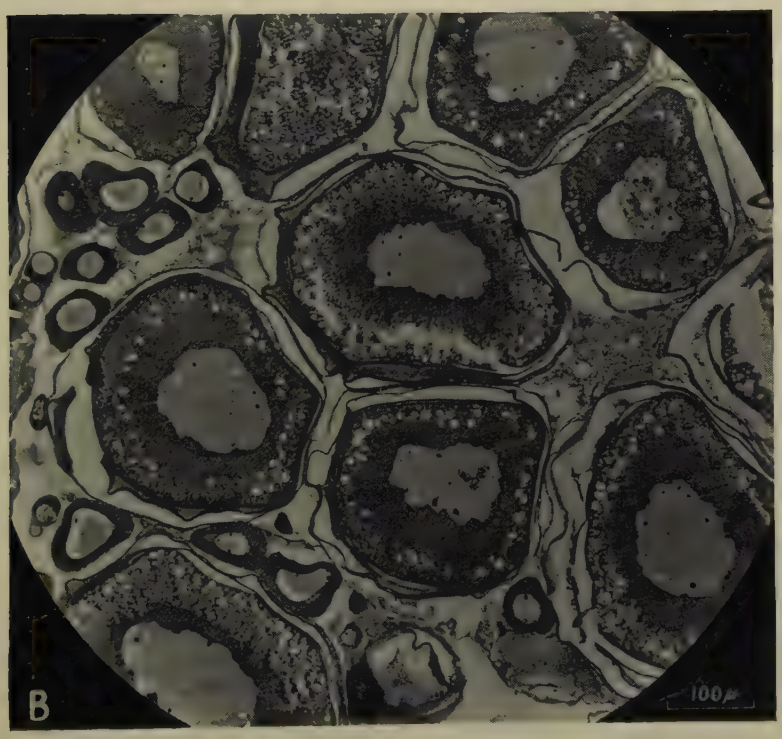
EXPLANATION OF PLATE I

Sections through the right ovary of Arctic cod

A. Summer resting condition (August) showing (*a*) clusters of small oogonia, (*b*) small primary oocytes, (*c*) larger secondary oocytes in which cytoplasmic differentiation has occurred.

B. Maturing ovary (November–December) showing the beginning of yolk deposition in the secondary oocytes. Small groups of primary oocytes are also present.





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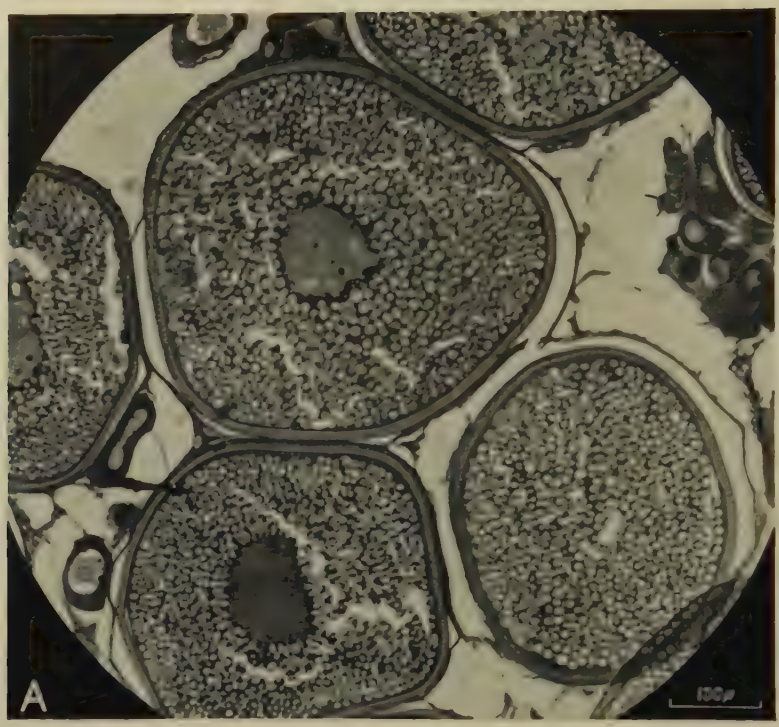
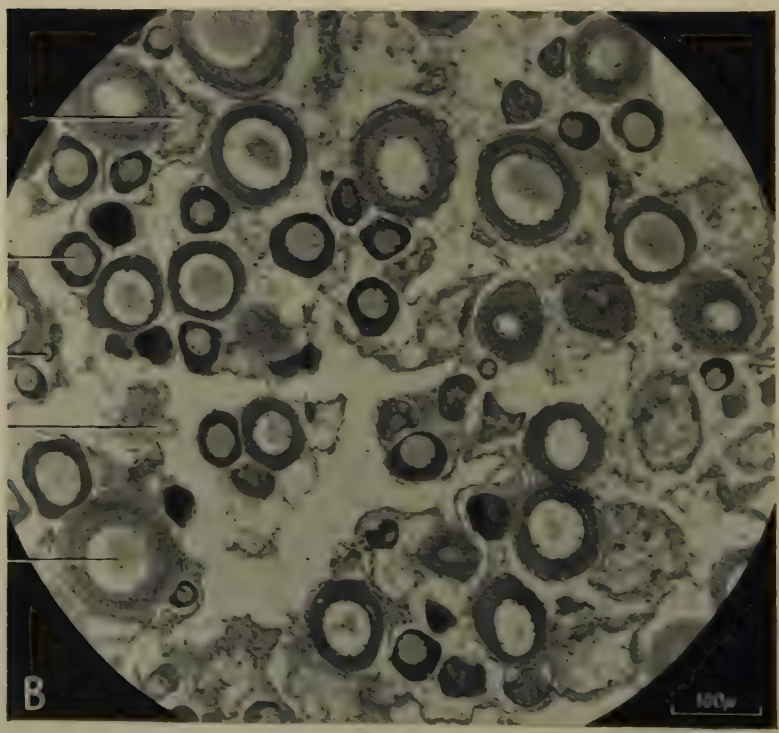

A
100μ


d
b
a
d
c
B
100μ

These oocytes remained in the ovary without undergoing growth or ripening during the next maturation cycle and formed the reserve stock of eggs which would mature for future spawnings. They began their growth in the spent fish. In addition, there was a second group of larger, secondary oocytes ranging in size between 80 and 170 μ in which ripening had begun. These cells stained lightly, and cytoplasmic differentiation had occurred; at the periphery of the cell, there was usually a distinct area in which the cytoplasm appeared vacuolated and less dense. These secondary oocytes matured to form the ripe eggs for the next spawning. The lobular structure of the ovary was distinct at this stage.

TABLE 2. MEAN EGG DIAMETERS IN THE OVARIES OF ARCTIC COD THROUGHOUT THE MATURATION CYCLE

Month	Ovary condition	Mean egg diameter (μ)	
		Primary oocytes	Secondary oocytes
August	Resting	60	130
October	Maturation begun	60	200
November–December	Yolk formation beginning	60	300
January	Ripening	60	420
Late February–March	Fully ripe: spawning	60	580
Late April–May	Spent: resorbing and regenerating	50	130
June	Resting	60	130

Maturation began in late September–October, when growth of the larger oocytes was resumed. There was initially a rapid increase in cytoplasmic volume, and the follicle surrounding the individual eggs became distinct. In November, some time after this phase, the first stages of yolk deposition were seen (Pl. I B). Small droplets of yolk, which stained light orange with Masson's trichrome stain, appeared at the edge of the cytoplasm, and gradually coalesced to form larger yolk globules. As the globules increased in size, their staining reaction intensified to a bright orange. The first indications of yolk deposition usually occurred in eggs of about 300 μ diameter, and in eggs measuring 400 μ large yolk droplets had formed (Table 2). As maturation proceeded and the eggs increased in diameter, yolk was gradually deposited throughout the entire cytoplasmic area. The nucleus underwent final changes at this time; it had a fine granular appearance and the nuclear membrane became indistinct; nucleoli were often present scattered throughout the nucleus. Fully mature

EXPLANATION OF PLATE II

Sections through the right ovary of Arctic cod

A. Ripe ovary (February–March) showing large eggs packed with yolk globules. Some small primary oocytes are also present.

B. Spent ovary (April) showing (*a*) small oogonia, (*b*) primary oocytes, (*c*) secondary oocytes, (*d*) resorbing follicles.

ovaries were taken from cod caught on the Norwegian coast spawning grounds in February. The mature eggs (Pl. II A) ranged in size from 500 to 650 μ, the average diameter being 580 μ (Table 2). Between the large ripe eggs, clusters of the small reserve eggs were found.

In sections of the spent ovary, the ruptured follicles, which had contained the ripe eggs, were abundant (Pl. II B). Resorption of the follicle remains began almost immediately after spawning, and unshed ripe eggs remaining in the ovary were also broken down and absorbed. At this time there was also a proliferation of the primary germ cells in the ovary, and new nests of small oocytes were formed. Simultaneously, the oocytes remaining from the previous year began to grow, and cytoplasmic differentiation occurred. This growth and cytoplasmic differentiation of the small eggs was often well advanced while the ovary still contained a large number of resorbing follicles. The lobular structure of the ovary was reformed. The ovary then became quiescent and remained in this condition until maturation began again the following autumn.

Vitamin A aldehyde

Both the total amounts per pair of ovaries (Text-fig. 3) and the concentration (Text-fig. 4) of vitamin A aldehyde reached a maximum in February. The mean value for the concentration was higher in February 1959 than in February 1960, but the mean values for the total amount per pair of ovaries were similar in the two years. This last finding is undoubtedly fortuitous, since the range of the individual values for February 1960 was very great, the highest being over ten times greater than the lowest. As the percentage of dry matter and the weight of the ovaries increased in November 1959 there was an increase in the content of the aldehyde. In the months May–August, when the ovaries were in a resting state, no vitamin A aldehyde was detected, and probably none was present in April and September.

The contents of vitamin A_1 and A_2 aldehyde of the ovaries from fish caught in February 1960 were determined. The mean total concentration was 3·91 μg/g dry matter, of which 27 % was vitamin A_2 aldehyde.

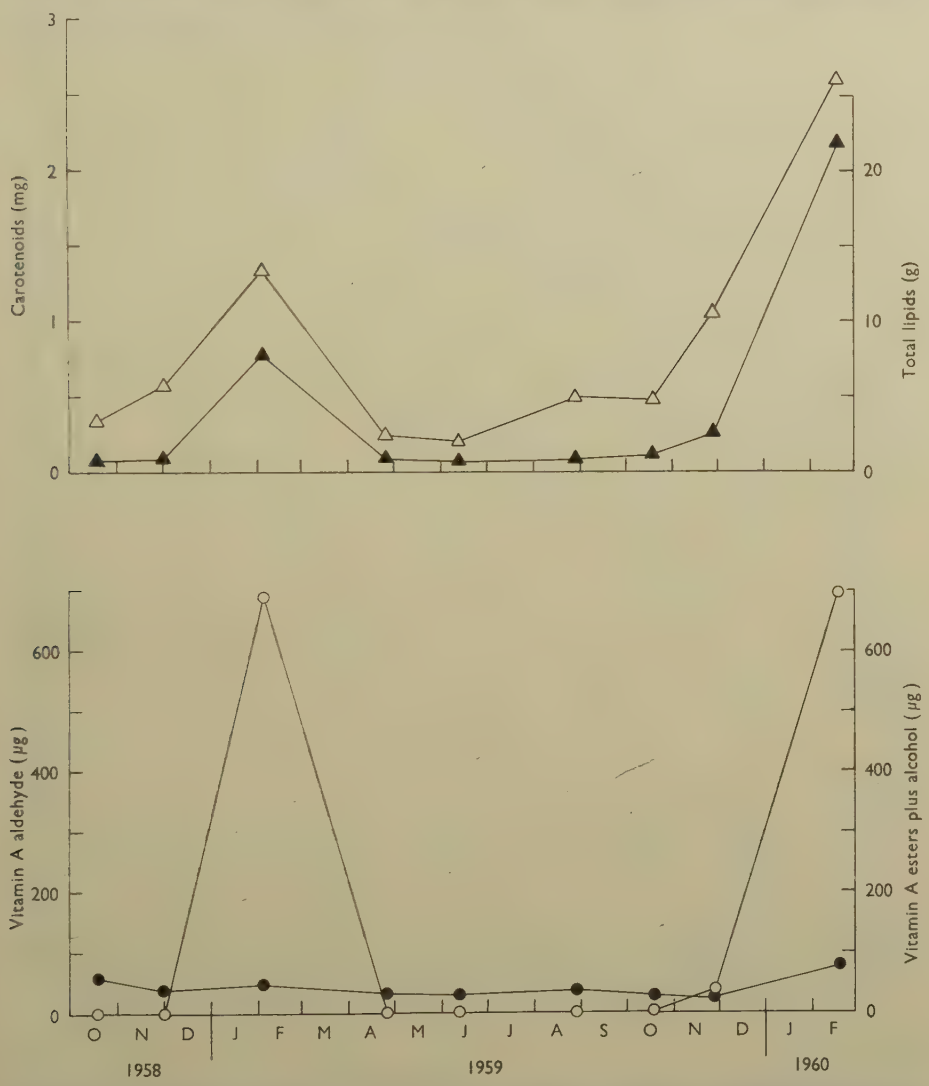
Vitamin A esters and alcohol

Text-figs. 3 and 4 show the variations in the total amount and concentration of the ester and alcohol forms of vitamin A. From the concentration curve (Text-fig. 4) it will be seen that there were minimum concentrations in February of 1959 and 1960, but when the total amounts were plotted these minima became maxima. It follows, therefore, that the content of vitamin A esters and alcohol increases as the weight of the ovaries increases, but the two increases are not proportional. The extracts from the ovaries of fish caught in February 1960 were tested for vitamins A_1 and A_2, but the concentrations

were too low to determine the two forms, and too low, even, to be certain that vitamin A_2 esters and alcohol were present.

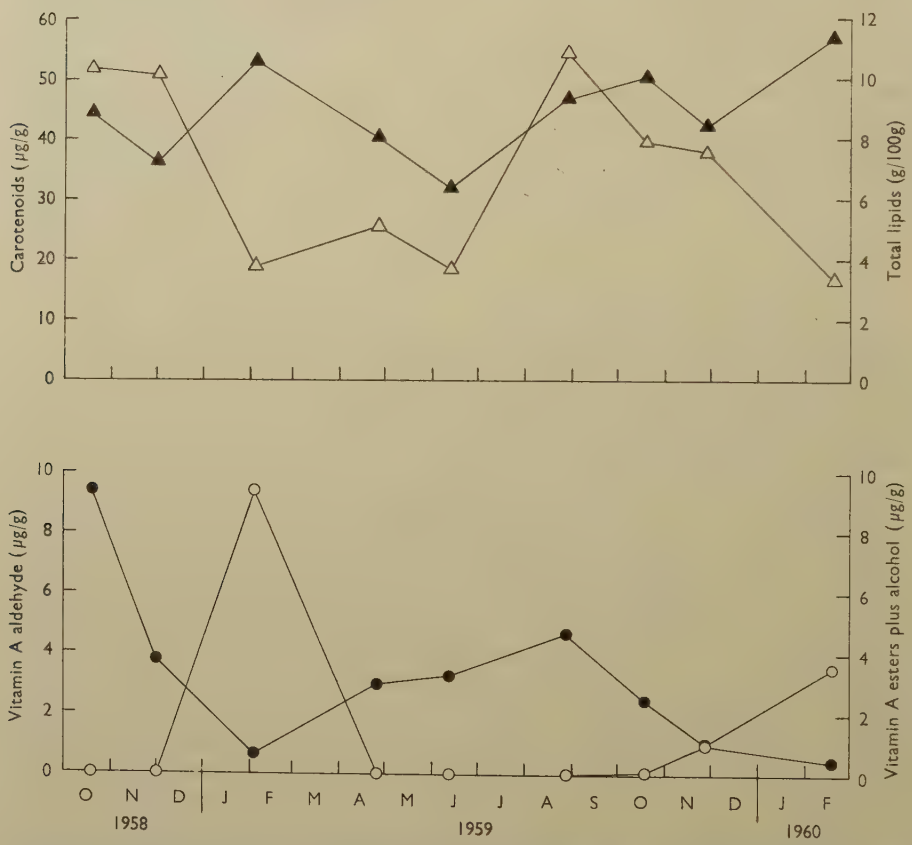
Carotenoids

The carotenoids present in the ovaries were not investigated thoroughly, but were estimated to obtain a rough indication of the quantity present in



Text-fig. 3. *Gadus morhua* from the Arctic. Mean values per pair of ovaries for the content of vitamin A aldehyde (○—○), vitamin A esters + alcohol (●—●), carotenoids (△—△), and total lipids (▲—▲) during the maturation cycle.

comparison with the vitamin A aldehyde. In the fully ripe ovaries, the total weight of carotenoids present was between two and four times the total weight of aldehyde. Most of the extracts gave absorption curves with single broad maxima at about 475 mμ, and the chromatographic behaviour of the pigments suggested that free and esterified astaxanthin were the principal carotenoids present. Text-fig. 3 indicates that carotenoids were deposited in the ovaries as they developed, but the concentration of carotenoids (Text-fig. 4) was found to be highest in August–October, before the ovaries increased in weight.



Text-fig. 4. *Gadus morhua* from the Arctic. Mean values for the concentration on a dry-matter basis of vitamin A aldehyde (○—○), vitamin A esters + alcohol (●—●), carotenoids (△—△), and total lipids (▲—▲) in the ovaries during the maturation cycle.

Lipids

Both the total amount and concentration of lipids in the ovaries were highest in February (Text-figs. 3 and 4), as would be expected if lipids were laid down in the ripening eggs.

DISCUSSION

This study has many imperfections, some of which are inherent in work on the Arctic cod, though others might have been avoided. From the preliminary results of 1958 and early 1959 it was obvious that in the succeeding winter more samples between November and April were required, since this was the period when vitamin A aldehyde appeared in the ovaries. Unfortunately, collection of samples for this work had to take second place to the other duties of the research vessel, and the samples for the winter of 1959–60 were collected on dates near to those of the previous winter. For the results to be strictly comparable, the samples should be from fish of the same race and the same age, allowance being made for the increase in age during the study. The first condition has been satisfied and an attempt has been made to fulfil the second by collecting samples from cod of such sizes as to allow for the annual growth cycle during the two periods of gonad maturation studied. The problem of storage has to be faced with all biological material brought from distant waters, and deep-freezing was decided upon, although it is not a perfect method, and there is some loss of vitamin A compounds on storage in deep-freeze. Added to these problems was the finding that in the ovaries of fish caught in February 1960 some 27% of the total vitamin A aldehyde was in the form of vitamin A_2 aldehyde. If this proportion of vitamin A_2 aldehyde had been present in the other ovaries analysed, then the values given in Text-figs. 3 and 4 would only represent about 90% of the total vitamin A_1 and A_2 aldehyde content. The error is not so great as the proportion of the two aldehydes would indicate, since in the Carr–Price test the blue colour produced by vitamin A_2 aldehyde has a maximum at 741 mμ but still has a considerable absorption at 664 mμ, which would have been measured in the routine method. For this reason, when vitamin A aldehyde has been recorded as absent, neither the A_1 nor the A_2 aldehyde was present.

The main finding of the investigation is not affected by these imperfections. It is that vitamin A aldehyde is found in cod ovaries only when they are maturing and contain large ripening eggs. Of the samples taken, those of February contained the highest concentration; some of the cod caught at this time were tagged and liberated, and recaptures on the spawning grounds showed that they were about 2–6 weeks from spawning. In October 1960, one pair of ovaries out of ten contained some aldehyde and in November 1960, six pairs out of eleven contained aldehyde. Coinciding with this occurrence of aldehyde the mean weight and percentage of dry matter of the ovaries were increasing, and the eggs growing in size. When the ovary was in the resorbing spent condition in April, or in the resting state from June to September, no aldehyde could be detected. It was not so with the other substances, vitamin A esters and alcohol, carotenoids and lipids, all of which

were present in resting ovaries, although they all increased in total amount to a maximum in February.

The difference between the mean ovary weight in February 1959 and February 1960 was due partly to the fish being larger in 1960 and partly to the fact that they were caught 2 weeks later in the year, and hence were nearer to spawning than those of February 1959.

Brækkan (1958), in a study of vitamins of the B group and the reproductive cycle of ovaries in cod, found that the percentage of dry matter increased from 18·3% at the first stage of regeneration of the spent ovary to 26·7% for fully ripe ovaries. Our findings are in good agreement with these figures.

Essentially, the maturation cycle of eggs within the ovary was being followed in this study. During the period of ripening, vitamin A aldehyde, vitamin A esters and alcohol, carotenoids and lipids are all being laid down in the eggs, as is shown by the fall in total amount of all these substances in the ovaries after spawning (Text-fig. 3). With vitamin A esters and alcohol, and carotenoids, however, although the total amounts increase during egg development their concentration decreases, but increases again after spawning, so that the concentration in the ovary tissue and small immature eggs must be greater than that in the ripe eggs. The results confirm the previous finding (Plack *et al.*, 1959) that some ten times as much vitamin A aldehyde is laid down as vitamin A esters and alcohol, but they also show that the quantity of carotenoids in ovaries with ripe eggs is some two to four times greater than that of vitamin A aldehyde.

No conclusions can be drawn about the origin of the vitamin A aldehyde. The question of whether it is transferred as such from other tissues to the ovaries, or whether it is formed in the ovary tissue itself from a precursor, cannot be answered from the results of this investigation. However, the aldehyde was absent during the early part of the maturation process when the oocytes were increasing in size and only began to appear in small quantities in November–December. This appearance of aldehyde seemed to coincide with the histological evidence of the onset of vitellogenesis, and the vitamin A aldehyde may be incorporated in the ripening eggs with the yolk; as the yolk content of the egg increases rapidly during the later stages of ripening, the aldehyde content also rises. However, further experiments are required to show whether the aldehyde is deposited in the eggs continuously throughout vitellogenesis or only over a restricted period. If the latter were true, it might explain the differences in concentration found in February 1959 and February 1960. In February 1960 the ovaries were from cod caught more than 2 weeks later and were certainly larger and more ripe than in February 1959, and it seemed possible that the lower concentrations of aldehyde might be a dilution effect. Inspection of the individual results for February 1960, however, showed that the heavier ovaries did not have lower concentrations of aldehyde.

After spawning the lipids were at a lower concentration than in the ripe

ovaries, but although the ovaries then entered a resting condition, the lipid concentration had fallen further by June, after which it rose to a high level in August–October; the carotenoids and vitamin A esters and alcohol also increased in concentration between June and August. It seems likely that these changes in concentration in spring and summer, when there is apparently little gonadal activity, are associated with changes in feeding. There is little food available in the winter and spring around the Bear Island shelf (where the spring samples were taken), so that by the end of May the cod are thin, weak and very dull in colour when caught in the trawl, the skin is flaccid and the liver very small and highly discoloured by bile pigments, and many of the fish appear to be in a state of starvation. Cod caught in the summer, when feeding heavily, provide a marked contrast, being firm-skinned, brightly coloured, and very lively; the large cream-coloured liver now extends throughout the body cavity. The fall in concentration of lipids in the spring may be due to their being withdrawn from the ovaries in starving cod. The rise in their concentration, and that of carotenoids and vitamin A esters and alcohol, during the summer, is probably associated with the resumption of intensive feeding activity by the fish and the general deposition of metabolic reserves.

Little is known of the role of vitamin A compounds in maturation and in the embryonic development of fish, but in higher vertebrates a vitamin A deficient maternal diet leads to abortive or abnormal embryonic development. It seems likely that vitamin A compounds are also required for the normal development of the fish embryo, since Hubbs & Stavenhagen (1958) have shown that when female green-throat darters, *Etheostoma lepidum* (Baird and Girard), were given a diet deficient in vitamin A or carotenoids their eggs and larvae had only a short survival period, whereas addition of vitamin A to the maternal diet produced viable offspring. In addition, vitamin A aldehyde (retinene) is active in the visual cycle and it may be that the aldehyde in the eggs passes to the eyes of the cod larvae. The behaviour of newly hatched planktonic fish larvae is strongly influenced by light (Woodhead & Woodhead, 1955) in such a way that in the sea the larvae would be maintained at a depth where food organisms are abundant. As cod larvae also select their food visually from the plankton, the early survival of the young larvae may be largely dependent upon the acuity of the visual process.

We are grateful to Messrs Explorator Ltd for the transport of frozen samples.

SUMMARY

Cod (*Gadus morhua* L.) from the Arcto-Norwegian stock that spawns off northern Norway were caught at various times during their gonadal maturation cycle over a period of 16 months. The ovaries were removed and either fixed for histological work or deep-frozen for the determination of vitamin A compounds.

The ovaries were inactive from June to September. Maturation began in late September–October and fully ripe eggs were present in February–March, the range of sizes being 500–650 μ. After spawning there was a period of resorption and regeneration, until May, followed by the quiescent summer period.

During maturation, the weight of the ovaries increased and the percentage of dry matter rose from about 16 to 29%; the total amount of vitamin A aldehyde, vitamin A esters and alcohol, carotenoids and lipids in the ovaries also increased, though the concentrations of the carotenoids and vitamin A esters + alcohol on a dry-matter basis fell. Vitamin A aldehyde was only found in maturing ovaries and in February, just before spawning, the weight ratio of aldehyde to esters + alcohol was about 10 to 1. The other substances were present in resting ovaries. The appearance of aldehyde in the ovaries occurred when there was histological evidence for the onset of vitellogenesis.

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OBSERVATIONS ON *ECHENEIBOTHRIUM MACULATUM* (CESTODA: TETRAPHYLLIDEA)

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(Plate I and Text-figs. 1–30)

Forty-five specimens of *Raia montagui* Fowler were recently examined for their cestode parasites and twenty-seven of these contained *Echeneibothrium maculatum* Woodland, 1927, a species which has not been found in any other elasmobranch. In addition to its well-marked specificity to this host there is an indication that *E. maculatum* prefers to attach itself to a particular region of the intestine. The cestode was not recognized as a valid species by Baylis (1948) and is included as a synonym of *E. dubium* Van Beneden 1858 in the Plymouth Fauna List (Marine Biological Association, 1957), but Williams (1958*a*) and Euzet (1959) were not in agreement with these views. For this and other reasons a re-investigation of *E. maculatum* was undertaken.

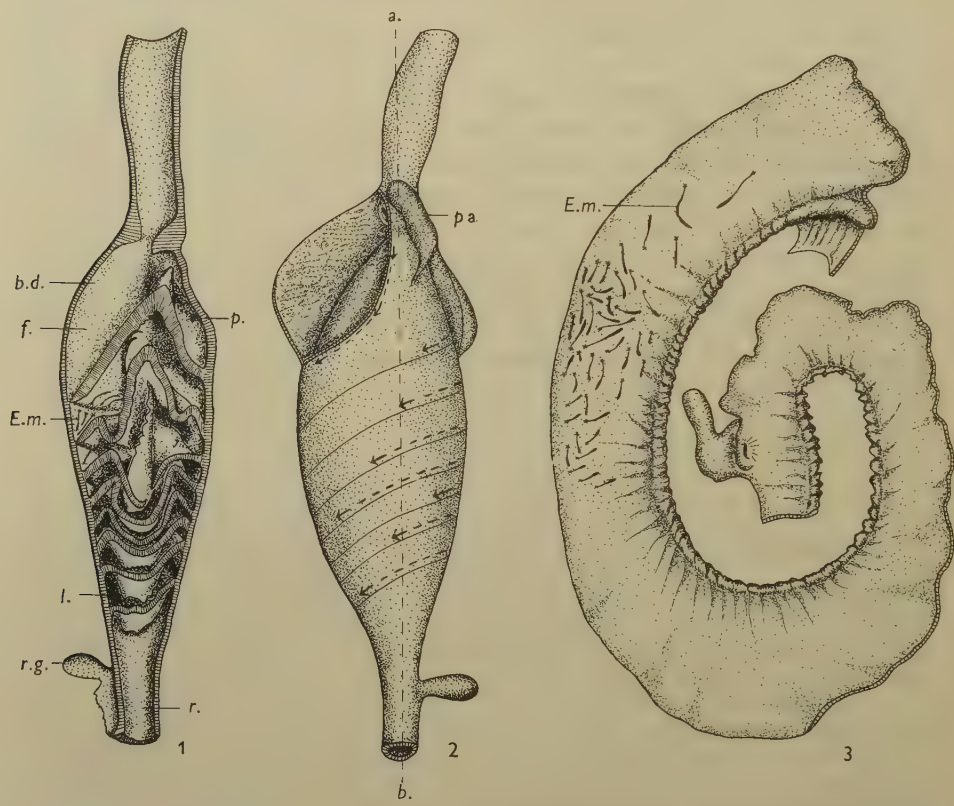
MATERIALS AND METHODS

The specimens of *Raia montagui* were trawled off Plymouth by R.V. 'Sula' during August and September, 1958 and 1959. The rays were kept alive in the large sea-water tanks outside the laboratory and were examined for their cestode parasites as soon as possible, usually within a period of 24 h. This was found to be necessary because elasmobranchs kept alive under these conditions, presumably without food, have shown a strong tendency to lose their intestinal parasites. It is hoped to discuss this phenomenon more fully elsewhere.

The intestines of some of the fishes were removed from the freshly killed hosts and opened with scissors following the course indicated by the arrowed broken lines in Text-figure 2. Having been opened in this manner they could be quickly examined as a flat piece of tissue for their cestode parasites (Text-fig. 3). A few intestines were preserved whole in 4% neutral formaldehyde and were examined at a later date using the method mentioned above or were sectioned vertically (Text-fig. 1) for a rapid determination of the exact location of *E. maculatum* within the gut.

Twenty-two male and twenty-three female specimens of *R. montagui* were examined and of these eleven males and sixteen females harboured *E. maculatum*. This parasite and the proglottides which it liberates were sometimes present in large numbers, but no record has been made of the exact number present in each fish. As an example of a heavy infestation, however, one small male ray, measuring 6 in. across the wings, harboured 123 strobilae and 503 proglottides. In addition other tetraphyllideans, including another species of

Echeneibothrium and members of the genera *Phyllobothrium*, *Acanthobothrium*, *Onchobothrium* and *Tritaphros*, were sometimes found in the fishes infested with *E. maculatum*. Woodland (1927) referring to *E. maculatum* stated that 'this small form is fairly common in *R. maculata* [=*R. montagui*] at Plymouth, it having been found in eighteen out of the forty-seven fish examined' and according to Baylis (1948), who examined seventeen specimens of *R. montagui*, *E. maculatum* is 'often present in hundreds'.



Text-figs. 1–3. Intestine of *Raia montagui* showing method used in determining location of *E. maculatum*. *a–b*, axis along which intestine was sectioned; *b.d.*, approximate position of bile duct opening into intestine; *E.m.*, *E. maculatum*; *f.*, first chamber of spiral valve intestine; *l.*, last chamber of spiral valve intestine; *p.*, approximate position of pancreatic duct opening into intestine; *pa.*, pancreas; *r.*, rectum; *r.g.*, rectal gland.

In order to study the anatomy of *E. maculatum*, living specimens were examined in a drop of sea water on a slide and, in some instances, the scolex was examined while still attached to a piece of intestine. The results obtained from living specimens were later confirmed by a detailed examination of material which had been fixed in 4% neutral formaldehyde, Bouin's or Gilson's fluids. Temporary mounts of fixed specimens were studied under a coverslip in water, lactophenol, or a solution of 5% glycerine in

alcohol. For permanent whole mounts Paracarmine, Ehrlich's haematoxylin or Acetocarmine were used as stains and the specimens cleared in xylene, clove oil or methyl benzoate. Unstained whole mounts were also prepared and found useful for an examination of the scolex while still attached to the gut. Some features of the anatomy of *E. maculatum*, for example, the cirrus spines (Text-figs. 29, 30) and the spines found on the scolex (Text-fig. 4) are well seen in mounts made in polyvinil-lactophenol, a method used by Thompson (1958) for an examination of the radula of *Adalaria proxima*. Serial sections cut in three planes, transverse, horizontal and sagittal, were stained with Mallory's triple stain, Heidenhain's haematoxylin with Ponceau S, or with Trioxyhaematin.

ECOLOGY

It has been found that *E. maculatum* appears to prefer a particular region of the intestine of *R. montagui* (Text-figs. 1, 3). Very few specimens were found in the first chamber of the spiral valve while the greatest concentration occurred in the second and third compartments. The number decreased in the fourth chamber and no specimens were found behind this region. Although the greatest concentration of attached strobilae was found in these locations the largest number of free proglottides always occurred in the anterior chamber of the intestine. This interesting phenomenon was also observed by Euzet (1959) in other tetraphyllideans and was recently confirmed by the writer. The best example appears to be the adult of *Orygmatobothrium musteli* from *Mustelus mustelus*, the scolex of which is attached to the posterior region of the intestine while large numbers of its proglottides accumulate in the anterior part of the gut. This suggests that an anterior migration of the proglottides occurs within the intestine but whether *E. maculatum* behaves in a similar manner is not known. It may be that in this latter species it is only those strobilae which are attached within the anterior chamber of the intestine that are responsible for liberating proglottides. The fact that the worms are invariably larger in size than those situated more posteriorly in the gut suggests that there may be a greater concentration of available food for them in this location or alternatively that the presence of fewer worms results in less severe competition for the same concentration of food. At present little is known of the metabolic requirements of Tetraphyllidea in relation to the production of proglottides and reproduction.

In addition to their tendency to concentrate in the second and third compartments, the specimens of *E. maculatum*, in the majority of the fishes examined, appeared to avoid attaching themselves near the central longitudinal food channel which runs through the intestine. This is interesting but perhaps not unusual behaviour as other parasites have been found to select well protected habitats, for example, Kabata (1959) obtained a similar result with parasitic copepods of the genus *Acanthochondria*.

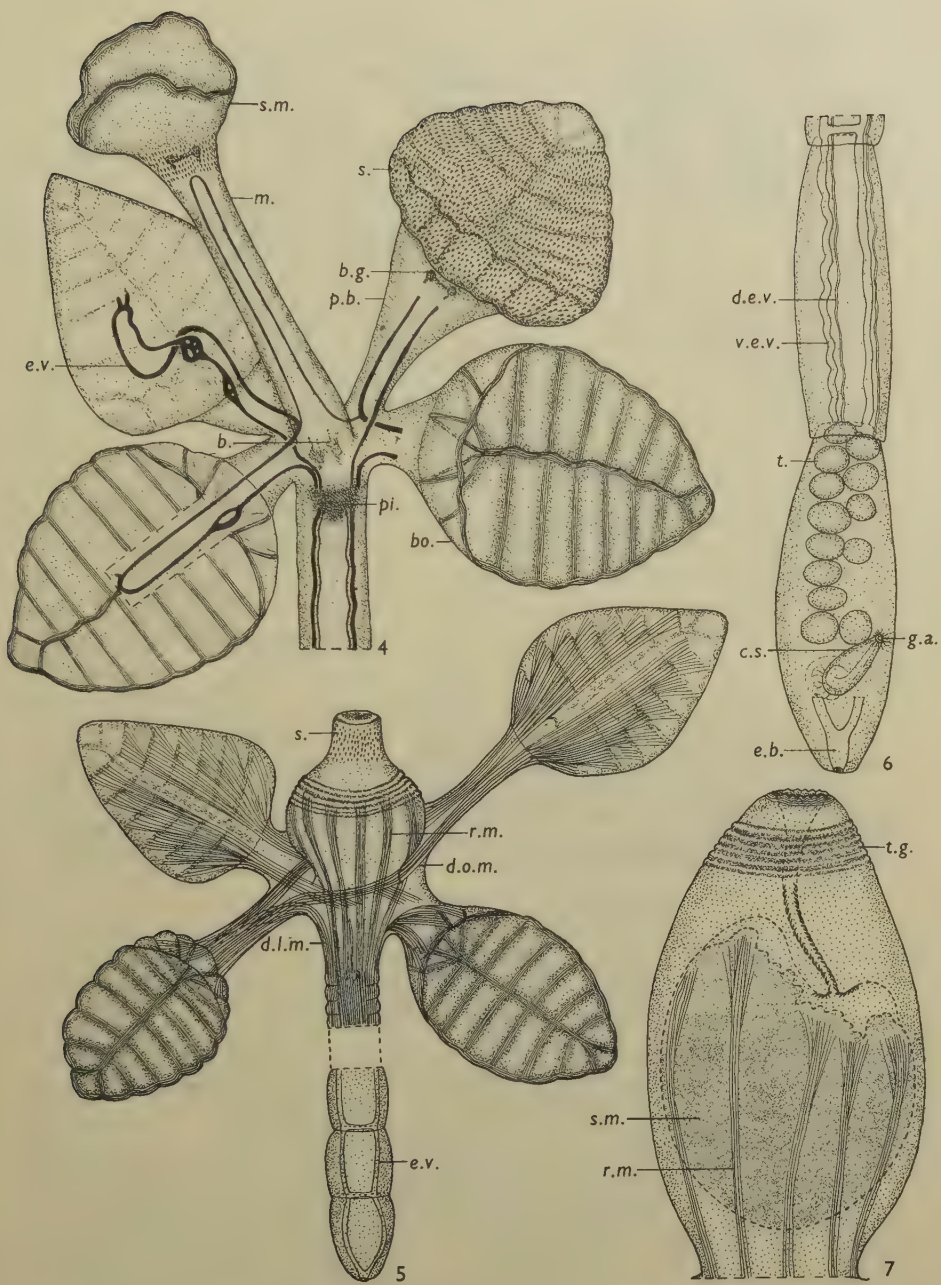
ANATOMY OF *ECHENEIBOTHRIUM MACULATUM*

External features of the scolex

The very brief accounts of the scolex given by Woodland (1927) and Euzet (1959) need supplementing.

The scolex is composed of a myzorhynchus and four pedunculated, spoon-shaped bothridia which are divided into loculi (Text-figs. 4, 11). It exhibits very little movement in living specimens that are still attached to the intestine (Text-fig. 8) but immediately becomes very active on being forcibly detached, the myzorhynchus and bothridia expanding and contracting continuously. The worms re-attach themselves readily to the intestine of a freshly killed *R. montagui*, the myzorhynchus as well as the apex of each bothridium appearing to function in the first instance as probing organs until a suitable position for attachment is found, and then as organs of adhesion. The bothridia of the scolex, while still attached to the intestine, cover an area of about 1·0 mm in diameter and the myzorhynchus may be 1·0 mm in length and 0·07 mm in diameter (Text-fig. 8). In detached specimens this latter organ may contract to 0·2 mm in length by 0·18 mm in diameter and assume a variety of shapes (Text-figs. 5, 7, 22) while the loculate surface of a bothridium measures about 0·5 mm in length and 0·25 mm in breadth. The above measurements were recorded from stained whole mounts.

It is often extremely difficult and sometimes impossible to determine the exact number of loculi within each bothridium when examining fixed stained specimens, but it has been observed, from living material, that there are usually eighteen but occasionally twenty loculi present. Woodland (1927) recorded only fifteen or seventeen loculi. Each bothridium possesses minute cuticular spines covering most of its convex or inner surface (Text-figs. 4, 12, 16), a feature not previously mentioned. Even smaller spines are present on the outer concave or loculate side of the bothridium (Text-fig. 12). It is not known whether these spines assist in attaching the parasite to the gut as no specimens have been found in which they were actually piercing or even closely adhering to the epithelial cells of the mucosa. Possibly they are defensive structures preventing the scolex from being crushed and therefore damaged when the folds of the mucosa move towards each other during peristalsis which has been observed to be extremely active in freshly killed rays. The presence of minute cuticular spines is a very characteristic feature of the scolex in other members of the genus *Echeneibothrium* but most previous descriptions do not mention this feature. The presence of deep corrugations or grooves on the inner or convex surface of the bothridium (Text-fig. 17) is another interesting but previously unrecorded character of *E. maculatum*. These may be a provision to allow for expansion of the body wall without the actual stretching of the cuticle which may not be as readily expansible as the unstriated muscles within the bothridium. Manton (1959) is of the opinion



Text-figs. 4–7. *Echeneibothrium maculatum.*

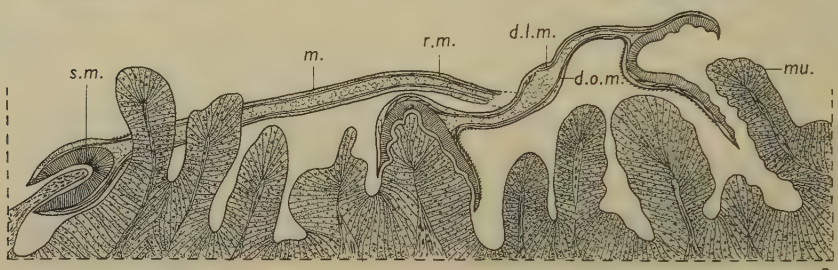
Fig. 4. Scolex of a mature individual in which are shown parts of the nervous and excretory systems.

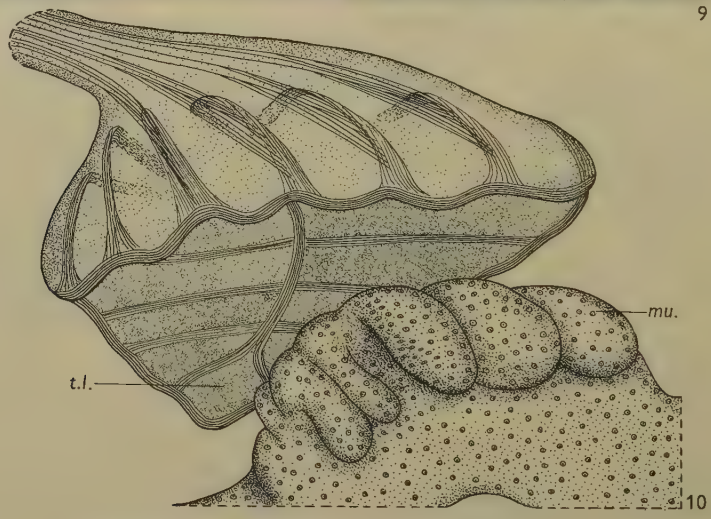
Fig. 5. Scolex of an immature specimen with twelve proglottides, drawn to same scale as Fig. 4.

Fig. 6. Posterior proglottides of an immature individual showing only thirteen testes.

Fig. 7. Contracted myzorhynchus with retracted sucker.

b., brain; *b.g.*, bothridial ganglion; *bo.*, bothridium; *c.s.*, cirrus sac; *d.e.v.*, dorsal excretory vessel; *d.l.m.*, deep longitudinal muscles; *d.o.m.*, deep oblique muscles; *e.b.*, excretory bladder; *e.v.*, excretory vessel of bothridium; *g.a.*, genital atrium; *m.*, myzorhynchus; *p.b.*, peduncle of bothridium; *pi.*, red 'pigmented' region; *r.m.*, retractor muscles; *s.*, spines; *s.m.*, apical sucker of myzorhynchus; *t.*, testis; *t.g.*, transverse grooves; *v.e.v.*, ventral excretory vessel.





Text-figs. 8–10. *Echeneibothrium maculatum.*

Fig. 8. Showing orientation of bothridium and myzorhynchus while attached to the mucosa of the intestine.

Fig. 9. Section through scolex attached to intestine.

Fig. 10. Showing natural position and shape of bothridium when attached to a portion of the mucosa which, in this case, shows a distinct imprint of some of the posterior loculi.

d.l.m., deep longitudinal muscles; *d.o.m.*, deep oblique muscles; *m.*, myzorhynchus; *mu.*, mucosa; *r.m.*, retractor muscles; *s.m.*, apical sucker of myzorhynchus; *t.l.*, anterior terminal loculus of bothridium.

that a furrowed cuticle may serve this function in some arthropods. The myzorhynchus is an extremely variable organ capable of extending to at least five times the length it assumes when fully contracted. Furrows or grooves are present in its body walls (Text-figs. 7, 22), which again may enable the organ to reach considerable length in its search for a suitable area to attach itself.

Woodland (1927) mentioned briefly the extreme variability in the length of the myzorhynchus and described the extremity of this organ as a 'thick pad covered with a circular fold of the terminal subcuticula save for a small central opening' (see Text-fig. 9) and stated that it resembled a sucker when retracted. The presence of a powerful functional sucker at the apex of the myzorhynchus is confirmed; but it may be completely withdrawn into the organ (Text-figs. 7, 9, 20–23) by means of powerful retractor muscles. When the myzorhynchus is fully expanded conspicuous cuticular spines are clearly visible in the region immediately behind the apical sucker (Text-figs. 4, 22). These spines, not mentioned in previous descriptions, may become inverted and lie within the myzorhynchus when it is fully contracted (Text-fig. 7).

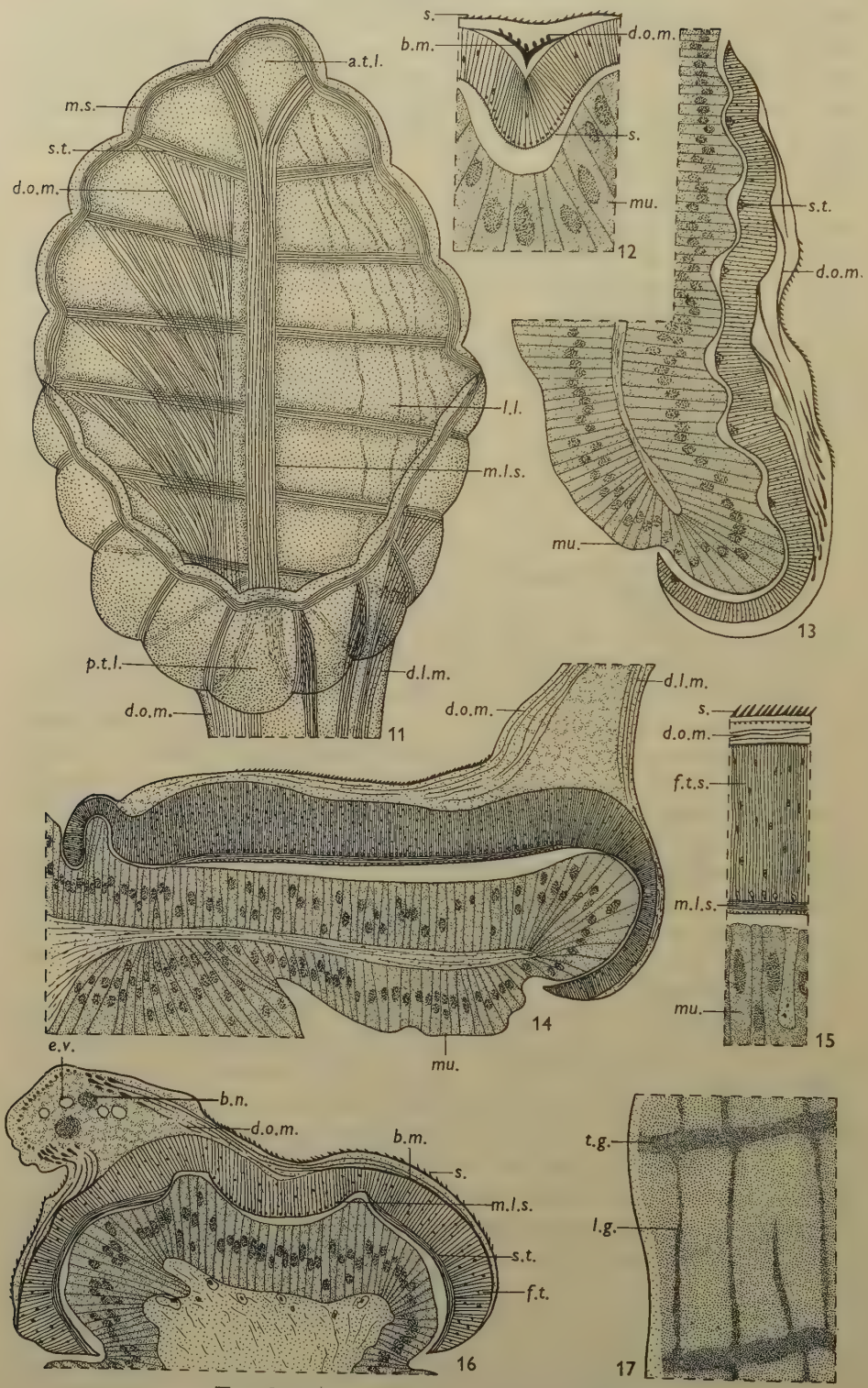
Musculature

Accounts of the main groups of muscles to be found in Tetraphyllidea have been given by Rees (1946, 1953) for *Phyllobothrium dohrnii* and a species of the genus *Echeneibothrium*, respectively.

The present description, therefore, will deal mainly with the musculature of the scolex because, so far as I am aware, a study of this system in relation to the function of the scolex has not been previously investigated in the genus.

The musculature of the strobila will not be described, as adequate descriptions are available. Woodland (1927) noted in *E. maculatum* 'the absence of a distinct internal layer of longitudinal muscle fibres and bundles of fibres throughout the subcuticular layer'. The writer's observations on transverse sections, however, indicate that muscle fibres are present, but are difficult to detect at the posterior end of the strobila and in the free proglottides, possibly due to the expansion of the uterus on becoming filled with eggs. That powerful longitudinal muscles are present is clearly indicated by the continuous contraction and expansion of living worms and proglottides.

When viewed in longitudinal and transverse sections it becomes evident that the bothridium functions as a large shallow sucker enveloping the host's epithelial tissue (Text-figs. 14, 16). The size of the bothridial opening appears to be controlled by muscles which may be derived from the longitudinal superficial muscle fibres of the body wall, these having become concentrated around the margins of the opening (Text-fig. 11, *m.s.*). The main cavity of the bothridium is divided into eighteen or twenty loculi which result from a particular arrangement of the superficial musculature in its wall. There is a distinct group of median longitudinal superficial muscle fibres (*m.l.s.*) which divides



Text-figs. 11–17. For legends see opposite page.

the bothridium into two equal halves. At each extremity of the bothridium this group of longitudinal muscle fibres diverges into two and the fibres become attached to the basement membrane of the body wall, thus forming two terminal loculi (*a.t.l.*, *p.t.l.*). Each half of the bothridium is further subdivided by seven or eight groups of transverse superficial muscle fibres (*s.t.*). This arrangement appears to be the result of a concentration of the circular muscle fibres in particular regions of the body wall resulting in the formation of eight or nine lateral loculi (*l.l.*). The ends of these muscle fibres (Text-fig. 16, *s.t.*) are attached to the basement membrane in the wall of the bothridium. The effect of the above arrangement of the musculature within the bothridium is to form eighteen or twenty subsidiary 'suckers' or loculi, each of which envelops a small portion of the host tissue that is covered by the whole bothridium. A contraction of the muscles which form the loculi would obviously result in a firmer grip of the tissue. The effect of such an attachment of each loculus or 'sucker' can be seen in Text-fig. 10, the mucosa of the intestine showing an imprint of the posterior loculi of a bothridium.

The deep longitudinal muscle fibres at the base of the scolex of *E. maculatum* are arranged in a uniform ring (Text-fig. 8, *d.l.m.*), but on entering the scolex some spread out into the peduncles of the bothridia (Text-figs. 5, 9) and others into the myzorhynchus. In this latter organ the musculature becomes arranged into about thirteen separate bundles each containing seven to twelve muscle fibres (Text-fig. 19, *r.m.*). These muscle bundles form the retractor muscles of the apical sucker of the myzorhynchus (Text-figs. 20, 23). They function in withdrawing the sucker into the myzorhynchus (Text-fig. 7). The length and diameter of the myzorhynchus is probably controlled by the superficial circular and longitudinal muscles of its body wall.

The deep longitudinal muscle fibres, after entering the bothridium, are arranged in distinct bundles. The muscle fibres of each bundle spread out fanwise within the bothridium, their endings becoming attached to the base-

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Legends to Text-figs. 11–17.

Echeneibothrium maculatum showing the anatomy of a bothridium.

Fig. 11. Ventral view of bothridium showing loculi and main musculature.

Fig. 12. Portion of Fig. 13 in greater detail showing thickened basement membrane.

Fig. 13. Lateral longitudinal section through bothridium attached to the gut.

Fig. 14. Median longitudinal section through bothridium.

Fig. 15. Portion of Fig. 14 in greater detail.

Fig. 16. Transverse section through bothridium.

Fig. 17. Part of convex or dorsal side of bothridium showing grooves in the cuticle.

a.t.l., anterior terminal loculus; *b.m.*, basement membrane; *b.n.*, bothridial nerve; *d.l.m.*, deep longitudinal muscles; *d.o.m.*, deep oblique muscles; *e.v.*, excretory vessel; *f.t.*, fibrous tissue; *l.g.*, longitudinal groove; *l.l.*, lateral loculus; *m.l.s.*, median longitudinal superficial muscles; *m.s.*, marginal superficial muscles; *mu.*, mucosa; *p.t.l.*, posterior terminal loculus; *s.*, spines; *s.t.*, superficial transverse muscles; *t.g.*, transverse groove.

ment membrane of the fibrous tissue layer. It is interesting to note that the muscle fibre endings of each bundle are attached in a particular manner and region, that is to considerably thickened areas of the basement membrane which are situated transversely to the long axis of the bothridium, to the inner side of the fibrous tissue layer and between the loculi.

There is also present a system of deep oblique muscles (Text-figs. 5, 11, *d.o.m.*), the bundles of fibres crossing one another in the centre of the scolex (Text-fig. 21, *d.o.m.*) and then extending into the peduncles of the bothridia. This system of muscles, like the deep longitudinal muscles, becomes attached in particular regions to thickened areas of the basement membrane (Text-figs. 11, 16, *d.o.m.*).

A consideration of the structure in relation to the natural position of the bothridium when attached to the host's tissue (Text-figs. 8, 9, 14) shows quite clearly that a contraction of the deep longitudinal and deep oblique muscles would result in the withdrawal of the bothridium from the mucosa.

Nervous system

The nervous system of *E. maculatum* has not been described previously although it exhibits some interesting features. There is a prominent 'brain' (Text-figs. 4, 18) giving off nerves to the strobila, bothridia and myzorhynchus. The 'brain' is in the form of two bilobed ganglia connected by a transverse commissure and is centrally situated at about the level of the junctions of the peduncles of the bothridia with the scolex (Text-fig. 21). From each ganglion of the 'brain' a pair of lateral longitudinal nerves extends posteriorly to run the length of the strobila (Text-fig. 18, *l.n.*). A pair of nerves arises from each of the four lobes of the 'brain' to extend into the four bothridia (*b.n.*) slightly posterior to the junction of the peduncle and bothridium. The two ganglia in each peduncle are joined by a ring commissure (*b.c.*). In addition four nerves arise from the brain (Text-fig. 18, *m.n.*) and after extending anteriorly almost as far as the sucker of the myzorhynchus join with a ring commissure which

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Legends to Text-figs. 18–23.

Echeneibothrium maculatum.

Fig. 18. Diagram of nervous system of the scolex.

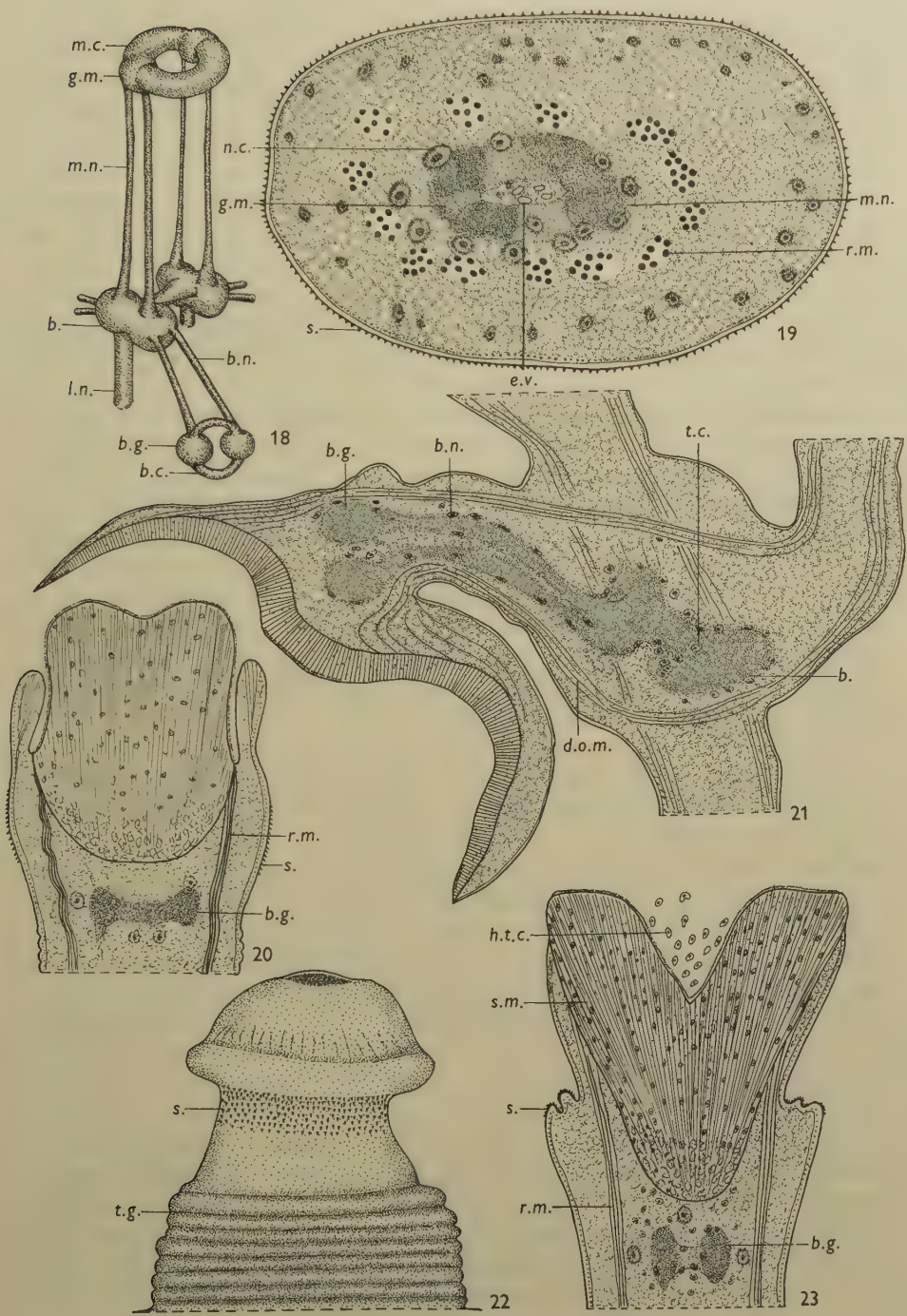
Fig. 19. Transverse section of the myzorhynchus showing the ganglia and commissure.

Fig. 21. Transverse section of the scolex in the region of the 'brain'.

Figs. 20, 22, 23. To show variation in the shape of the myzorhynchus and its apical sucker.

Figs. 20, 23. Median longitudinal sections. Fig. 22. Drawn from whole mount.

b., 'brain'; *b.c.*, bothridial commissure; *b.g.*, bothridial ganglion; *b.n.*, bothridial nerve; *d.o.m.*, deep oblique muscles; *e.v.*, excretory vessel; *g.m.*, ganglion of myzorhynchus; *h.t.c.*, host tissue cells; *l.n.*, longitudinal nerve to strobila; *m.c.*, circular commissure of myzorhynchus; *m.n.*, nerve to myzorhynchus; *n.c.*, nerve cell; *r.m.*, retractor muscles; *s.*, spines; *s.m.*, apical sucker of myzorhynchus; *t.c.*, transverse commissure of 'brain'; *t.g.*, transverse groove in cuticle of myzorhynchus.



Text-figs. 18–23. For legends see opposite page.

contains two swellings or ganglia (Text-figs. 18, 19, *g.m.*). It is of interest to note that conspicuous nerve cells (Text-fig. 19, *n.c.*) are associated with this nervous system, their structure and distribution bearing a marked resemblance to those described by Hadenfeldt (1929) in the turbellarian *Notoplana atomata*.

There is an indication that these nerve cells exhibit a definite arrangement in some regions of the scolex. For example, it has been observed from transverse sections of the myzorhynchus in the region of its ring commissure (Text-fig. 19) that a single nerve cell appears to be associated with each bundle of muscle fibres of the retractor muscle system of this organ. Further investigation is necessary to determine whether this interesting arrangement is constant. Andrew (1959) has already pointed out that nerve cells may be numbered in certain invertebrate phyla including the Acanthocephala and Rotifera.

In addition to these nerve cells which are closely associated with the nervous system, cells having a similar appearance occur immediately below the body wall of the myzorhynchus (Text-fig. 19). They have, therefore, been provisionally identified as nerve cells as they do not resemble subcuticular gland cells, which are often present in helminth parasites, and because of the extreme sensitivity of the myzorhynchus when touched. According to Hyman (1951, p. 253) the Cestoda, and in particular the Pseudophyllidea, lack special sense organs while the body surface and organs of the scolex are very well supplied with free sensory nerve cells and endings. The myzorhynchus in *E. maculatum*, as already mentioned, appears to function as a very efficient sense organ as well as one of attachment.

Excretory system

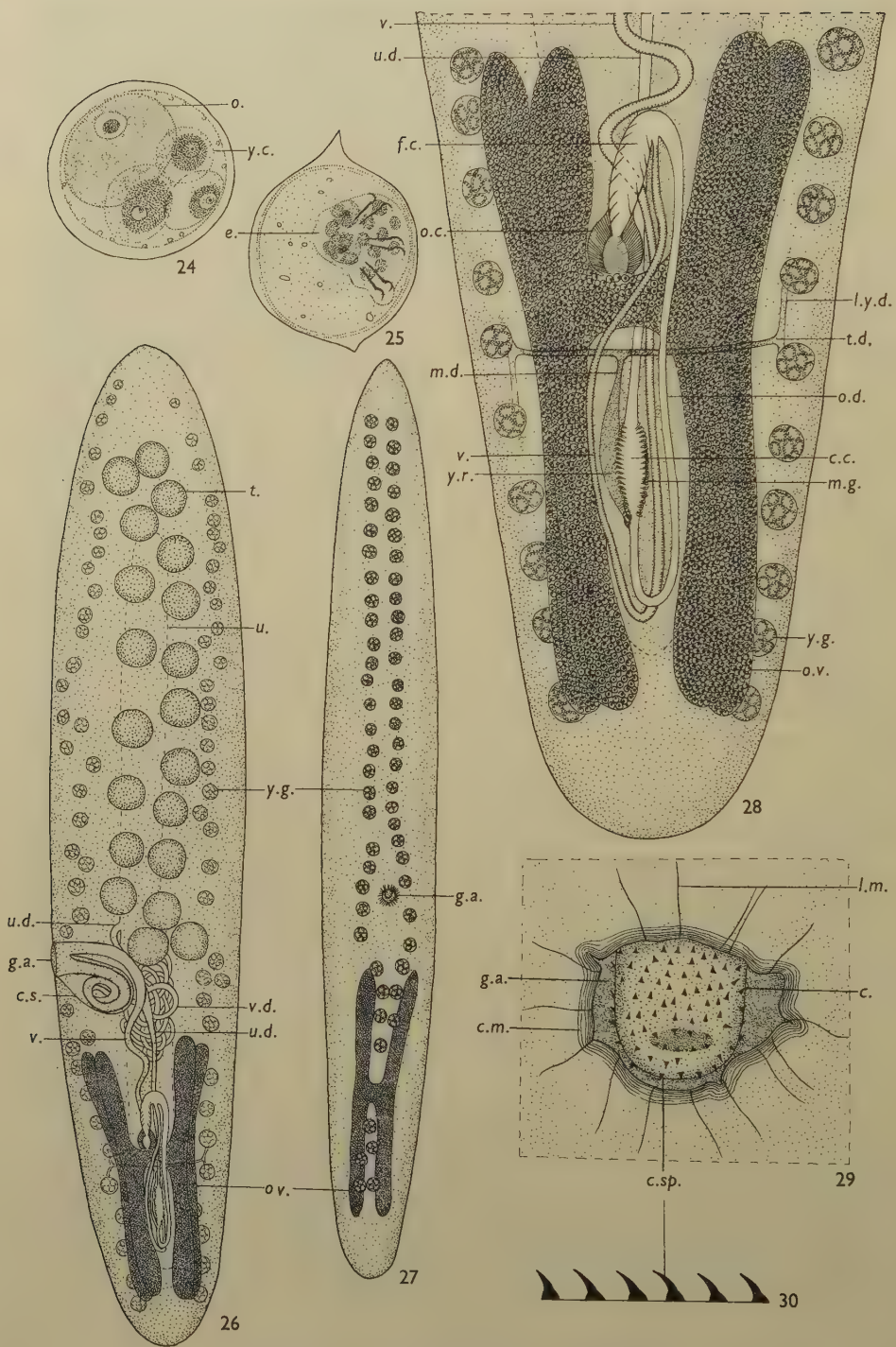
In general the excretory system resembles that described in other members of the Tetraphyllidea. Each bothridium is supplied with vessels which are continuous with those of the dorsal and ventral excretory vessels of the strobila. In the neck region the dorsal and ventral vessels are of the same diameter and extend into the dorsal and ventral bothridia respectively (Text-fig. 4). An excretory vessel also extends into the myzorhynchus. In some specimens it was seen that the excretory vessels anastomose within each bothridium, but further investigation is necessary to determine whether this is a constant feature.

Genital system

Echeneibothrium maculatum is protandrous, specimens measuring approximately 2 mm and having only eight distinct proglottides already possessing twelve testes and rudiments of the cirrus sac in the last segment of the strobila. It has been seen that specimens measuring 6 mm and with thirty proglottides possess twenty testes in the eighteenth proglottid. In addition these specimens showed distinct rudiments of the female genitalia in their twenty-fifth to

thirtieth proglottides. The total number of proglottides in strobilae showing every indication of being mature and of being in the process of liberating proglottides varies from 30 to 100, the latter number having been recorded in individuals 100 mm long. The average length and breadth of the posterior proglottid in fixed mature specimens is about 1·8 and 0·5 mm, respectively. Apparently the end proglottid becomes detached from the body soon after egg production has begun and the liberated proglottid may then continue to grow while it is free in the intestine, reaching a length of 4 mm and a diameter of 1 mm, these again being measurements of individuals fixed in 4% neutral formaldehyde. At this size the proglottides are fully distended with eggs as a result of the expansion of the uterus, and only vestiges of the male and female genitalia remain. As egg production progresses a degeneration has been noticed of the posteriormost testes of the proglottid. This is interesting because in immature individuals the first testes to appear are those in the posterior regions of the segments. It may be important to bear this in mind while using the number of testes as a taxonomic character. The ducts of the male and female genitalia open into a genital atrium, the pores being irregularly alternate and situated laterally, slightly in front of the posterior third of the proglottid (Text-fig. 26). The other features of the male and female genitalia are described separately below.

Male genitalia. There are usually twenty testes in each mature segment, but occasional specimens were found in which there were twenty-four, and in one liberated proglottid thirty-four were counted. The testes are arranged roughly in two rows almost meeting in the middle line, occupying two-thirds of the length of the proglottid and not usually extending behind the level of the cirrus sac (Text-fig. 26). They are spherical and situated dorsally to the uterus. No vasa efferentia have been seen, but the vas deferens which may be functioning as a vesicula seminalis is very long and coiled, occupying a dorsal position in the middle line, behind the testes and in front of the ovary. The vas deferens is continuous with a cirrus that is situated within the cirrus sac which measures 0·35 × 0·15 mm. The cirrus, which is well armed with minute spines (Text-fig. 30) measuring about 0·016 mm, opens into the genital atrium. According to Woodland (1927) the cirrus when fully extruded appears to exceed the length of the whole proglottid but the writer is unable to confirm this interesting observation. The cirrus when extended is certainly very long, a fact which may well have some connexion with the length of the vagina. No specimens were observed, however, in which the cirrus was inserted into the vagina of its own segment. This has been observed in some Tetraphyllidea, for example, *Phyllobothrium sinuosiceps* Williams, 1959, in which self-fertilization occurs. At present there is no evidence that self-fertilization occurrs in *E. maculatum*, but cross-fertilization of a liberated proglottid with another appears to be unlikely since, of the hundreds of proglottides which have been seen, none were united in copulation.



Text-figs. 24–30. For legends see opposite page.

Female genitalia. The ovary is situated posteriorly, lying behind the coils of the vas deferens. It is roughly H-shaped in dorsal, ventral and lateral views (Text-figs. 26, 27), as it possesses eight almost cylindrical branches projecting from a short transverse limb. The oviduct arises as a distinct ovicapt in the middle line from the anterior border of the transverse limb of the ovary and continues anteriorly in the form of a dilated valvular structure (Text-fig. 28) described as the 'fertilization chamber' by Woodland (1927). This may also function in preventing the ova from returning towards the ovary as a result of the suction pressure that is probably set up on the contraction of the powerful muscular wall of the ovicapt. The 'fertilization chamber' receives the vagina at its anterior border and then changes its course to proceed ventrally and backwards as the oviduct, almost as far as the posterior extremity of the ovary. Here it loops dorsally to take an anterior course and receives the median yolk duct immediately before dilating to form the dorsal central chamber or ootype surrounded by Mehlis's gland. The uterus emerges from the ootype as a slightly sinuous duct, which continues anteriorly in the mid-dorsal line to open, near the level of the genital pore, into the dorso-lateral side of the sac-like ventral uterus. No uterine pores opening to the exterior have been seen, the eggs being liberated into the sea presumably as a result of the rupture of the ventral body wall. This invariably occurs when fully gravid proglottides are placed in a vessel containing sea water.

From its external opening into the genital atrium the vagina takes a lateral course as far as the middle line where it turns sharply backwards. It runs posteriorly directly above the uterus as far as the 'fertilization chamber', becoming gradually dorsal in this region, and then continues backwards almost as far as the posterior extremity of the ovary. Here it loops ventrally taking an anterior course to open into the 'fertilization chamber'.

The yolk glands occur in the form of a double row of follicles on either side of the proglottid and extend along most of its length (Text-figs. 27, 28). The yolk ducts are first apparent as lateral longitudinal collecting tubules on either

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Legends to Text-figs. 24–30.

Echeneibothrium maculatum showing male and female genitalia.

Figs. 24, 25. Two stages in the development of an egg.

Fig. 26. Proglottid in ventral view (the outline only of the uterus is included).

Fig. 27. Another proglottid in lateral view to show the arrangement of the yolk gland.

Fig. 28. Posterior region of a proglottid to show the arrangement of the female ducts.

Fig. 29. Genital atrium and base of withdrawn armed cirrus.

Fig. 30. Spines from base of cirrus.

c., cirrus; *c.c.*, central chamber or ootype; *c.m.*, circular muscle; *c.s.*, cirrus sac; *c.sp.*, cirrus spines; *e.*, embryo; *f.c.*, 'fertilization chamber'; *g.a.*, genital atrium; *l.m.*, longitudinal muscle; *l.y.d.*, lateral yolk duct; *m.d.*, median yolk duct; *m.g.*, Mehlis's gland; *o.*, ovum; *o.c.*, ovicapt; *o.d.*, oviduct; *ov.*, ovary; *t.*, testis; *t.d.*, transverse yolk duct; *u.*, uterus; *u.d.*, uterine duct; *v.*, vagina; *v.d.*, vas deferens; *y.c.*, yolk cell; *y.g.*, yolk glands; *y.r.*, yolk reservoir.

side of the transverse limb of the ovary. In this region they join to form a transverse yolk duct from which the median yolk duct extends posteriorly, in the middle line. This latter duct is dilated to form a yolk reservoir before it opens into the oviduct in the neighbourhood of Mehlis's gland. Its opening appears to be surrounded by a small sphincter muscle (Text-fig. 28).

Some unforeseen problems have arisen in attempting to study the eggs and egg-shell formation in *E. maculatum* in relation to the structure of the genitalia. As far as can be ascertained at present, the eggs of the species when in an early stage of development are spherical in shape and possess an ovum surrounded by two or three yolk cells all of which are enclosed by at least two distinct membranes (Text-fig. 24). In some eggs, however, the ovum had already absorbed the contents of the yolk cells and had become a hexacanth embryo measuring about 0·013 mm in diameter (Text-fig. 25). As can be seen from this figure some of the eggs possess distinct processes or foldings on the outer shell wall. The eggs of *E. maculatum* measure 0·035 mm in diameter. According to Woodland (1927) in his specimens the eggs measured 0·055 mm, possessed six hooks, and were in 'clusters' when first deposited in the uterus. In other Tetraphyllidea from *R. montagui* it has been found that the eggs occur in 'clusters' and measure about 0·053 mm. It is possible therefore that Woodland may have been examining the eggs of a species other than *E. maculatum*.

A DISCUSSION OF THE SPECIFICITY OF *ECHENEIBOTHRIUM MACULATUM* TO *RAIA MONTAGUI*

Echeneibothrium maculatum appears to occur only in *R. montagui*, but according to Euzet (1959) a variety, *E. maculatum* var. *exiguum*, is sometimes present in *R. clavata*. As a result of an investigation of this 'variety' an account of which will be published at a later date, the writer is of the opinion that it may well be a distinct species. It is now thought that at least three main factors, namely, ecological, morphological and physiological, contribute towards restricting *E. maculatum* to one host species. Two of these factors are discussed briefly below. Although physiological factors cannot be considered here it is thought that advances in this field will add much towards an understanding of the host-specificity shown by these cestodes.

Ecological factors

Steven (1932) has shown that shoals of at least three members of the genus *Raia* are sometimes unispecific and possibly at certain times of the year unisexual. He also stated that there is some evidence for believing that the various species do not show extensive migratory movements. These observations are supported by Gerking (1959) who was of the opinion that many species of fish tend to remain in a particular area and even defend their territories against

intruders. If other species of *Raia* do not often enter areas occupied by *R. montagui* their chances of becoming infested with *Echeneibothrium maculatum* would certainly be diminished.

Little is known of tetraphyllidean life-histories, but from the available information it is reasonable to assume that *R. montagui* acquires *E. maculatum* by eating some animal which harbours the plerocercoid stage of the cestode. If this plerocercoid larva is also specific to the intermediate host then the food of *R. montagui* would be important in determining its cestode parasites. Euzet (1959) has already suggested that the plerocercoid stages of species of the genus *Echeneibothrium* may be specific to certain decapod crustacea of the order Anomura, but he also expressed the view that members of the genus *Raia* harbour different parasites, although their food is very similar. In addition, according to Steven (1947), rays are not specific in their feeding habits, but it was pointed out that little is known of this aspect of their biology.

In view of the alleged similarity in the food of rays it is possible that *R. montagui* ingests, while feeding, many species of the genus *Echeneibothrium*. A careful search of the intestine and its contents in freshly killed rays, however, suggests that *R. montagui* in Plymouth waters acquires in its food only two species of the genus *Echeneibothrium*, namely, *E. maculatum* and another species which may be *E. fallax*. The latter species shows every indication of being very unsuccessful in view of the number present, usually two to three specimens, and the immense host-tissue reaction to the parasite. *E. maculatum* on the other hand flourishes possibly as a result of its apparently very high degree of adaptation for attachment. So far there have been no indications whatsoever that larval stages of other species of *Echeneibothrium* are acquired by *R. montagui* and that these fail to establish themselves within the host. The above comments therefore suggest, but do not prove, that *R. montagui* is more selective in its feeding habits than hitherto believed. In addition it has been found that many species of the genus *Echeneibothrium* are specific to other rays and that according to the literature, as already mentioned, larval stages may well be specific to their intermediate hosts. Thus further detailed investigations should be made into the feeding habits of these fishes. Preliminary observations made at Plymouth support this view. For example, it was noted that polychaete worms, together with many crustaceans, formed an important part of the diet of twenty-one specimens of *R. montagui*, while no polychaetes were found in the stomachs of the twenty-five specimens of *R. clavata* that were examined. Fish remains, including many sand eels but no crustaceans or polychaetes, were found in the stomachs of seven specimens of *R. brachyura* and in three examples of *R. micro-ocellata*. Furthermore, according to Clark (1922), there were differences, although slight, in the stomach contents of young specimens of four species of *Raia*, and these variations are, in the writer's opinion, significant in investigations into the parasites of rays.

Differences in the structure of the mucosa of various rays have already been pointed out by Williams (1960) and work is now in progress on the possible correlation between these variations and the food taken by the fishes. The results from an investigation into this kind of relationship will, as Barrington (1957) and Andrew (1959) have pointed out, require a very careful analysis. The papers by Al-Hussaini (1949), Burnstock (1959) and Hinde (1959) indicate that a correlation may well occur between feeding habits and the structure of the intestine in some teleost fishes.

Some of the comments made above support the view expressed by Baylis (1938) that the habits, particularly as regards food and environment of the hosts, have played an important part in determining their helminth fauna. They also agree with the opinion of Prof. Jean Baer, who according to Cameron (1950) recognized two types of host-specificity, one depending on the phylogenetic relationships of the hosts and the other on their ecology.

Morphological factors

The greatest concentration of *E. maculatum* was found in the second and third chambers of the spiral valve intestine. It is possible that the cestodes are released in this location from the partially digested tissues of their intermediate hosts, as in many of the *R. montagui* that were examined a considerable amount of undigested food occurred in the first chamber of the intestine. Alternatively the larval cestodes may be liberated in other regions of the alimentary canal, in particular the stomach, and then undergo a migration or be carried to their normal habitat. It may be significant that the young stages of *E. maculatum* always contained, in their parenchymatous tissues, a high concentration of calcareous corpuscles and, as von Brand *et al.* (1960) have suggested, these may serve as a mechanism to counteract the high degree of acidity which is found in the stomach. The youngest specimens of *E. maculatum* found in the intestine of *R. montagui* indicated that the scolex of the parasite is in an advanced stage of development (Text-fig. 5) on infesting the host. The scolex of an adult worm (Text-fig. 4) which is liberating proglottides does not exhibit a significant difference in size and structure from that of the immature stage. In view of the fact that differences occur in the dimensions of the folds that constitute the mucosa of the gut as well as in the depths between these folds (Pl. I) it was thought possible that *E. maculatum* may seek an area of the gut to which it can attach itself most efficiently. For example, substantial variations occur in the depths between the folds at the anterior, middle and posterior regions of the intestine (Text-figs. 4–6), the average height of the primary folds measuring 1·10, 0·76 and 0·24 mm, respectively. However, since the average length of the loculate surface of the bothridium (in fixed specimens) is about 0·50 mm, it is not obvious why bothridia attaching themselves in the manner illustrated in Text-fig. 9 could not become attached to the mucosa of any of the three regions of the spiral valve. On the other hand,

it is evident that the size, shape and structure of the bothridium of this cestode appear to be perfectly adapted for attachment to this type of mucosa and not to an intestine like that of *R. naevus* where numerous villi are present. The proglottides of *E. maculatum*, the greatest concentrations of which occur in the anterior chamber of the intestine, would obviously find the greatest protection and possibly more nourishment in this position where the depth between the folds is at its maximum (Pl. I, fig. 4).

It is felt, therefore, that morphological factors of this nature should not be ignored in an explanation of the specificity of *E. maculatum* to *R. montagui*. As far as the writer is aware there are no previous accounts of morphological factors playing a role in the specificity of various tetraphyllideans to particular hosts and to precise habitats within the host. The above comments, therefore, have bearing on the remarks made by Shulman (1954) on the close contact and degree of morphological adaptation of the parasite to its host. He suggested that these may be significant factors affecting host-specificity which he regarded as one of the basic problems of contemporary parasitology. Llewellyn (1956) has also stated that in some of the Monogenea 'there is an exacting topographical relationship between parasite and host, and this is probably an important factor in the mechanism of host specificity'.

TAXONOMY OF THE GENUS *ECHENEIBOTHRIUM*

Euzet (1959) pointed out that the taxonomy of the Tetraphyllidea is in a state of chaos and made a valuable contribution towards solving many of the difficulties involved. Williams (1958*a*) in a preliminary account of some Tetraphyllidea from British elasmobranchs stated that little was known of the detailed structure of the scolex in *Echeneibothrium* and accepted *Rhinebothrium* Linton, 1890 as a synonym of the genus. Further work has now confirmed that in some species of *Echeneibothrium* the myzorhynchus may become completely retracted within the scolex, thus giving the impression that it is absent, but as already indicated by Williams (1958*b*) more detailed studies have now shown that *Rhinebothrium* is a valid genus.

Species of the genus *Echeneibothrium* found in British rays still present many taxonomic problems which, in the writer's opinion, can be solved only by further and more extensive work on the biology of these cestodes and their hosts, with due regard to the accurate identification of the fishes in which they are found, a task which is not always easy. Williams (1960) stated that some species of the genus *Raia* could be identified solely by an examination of the mucosa of the intestine and that occasionally *R. clavata* is confused with *R. montagui*. For example, a ray caught at Plymouth in August 1958 was provisionally labelled by the writer as 'either *R. montagui* or *R. clavata*, probably the former' but was only recently attributed to *R. montagui* after an examination of the intestine and its cestode parasites. In addition a recent

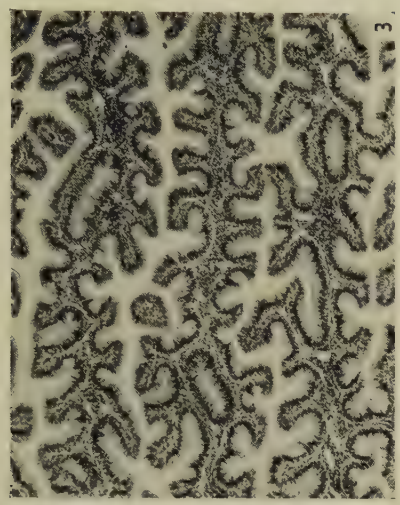
re-examination of the intestine of a ray revealed that it had been wrongly labelled as *R. montagui* when caught by the writer in the Irish Sea in the spring of 1957. The discovery that it was in fact *R. clavata* has contributed towards solving a problem regarding the parasites briefly described by Williams (1958*a*) as *E. fallax*. A cestode resembling *E. fallax* was present in this specimen of *R. clavata* but it occurred together with two other species of the genus *Echeneibothrium* both of which have now been found to be specific to this ray. The results obtained so far appear to indicate that in 'British' waters *E. fallax*, or a species with similar morphological features, is the only member of the genus which is not specific to a particular species of *Raia*. Further work is necessary either to confirm or disprove this suggestion. It is interesting to note, however, that the parasite now identified provisionally as *E. fallax* is never present in large numbers and stimulates a large amount of host-tissue reaction, facts which indicate that its scolex is not so well adapted for adhesion as it is in other members of the genus. The present investigation has shown that *E. maculatum* possesses 18–20 loculi in each bothridium, 20 testes in each proglottid, and that it parasitizes *R. montagui* only. It is, therefore, a valid species and, as already suggested by Williams (1958*b*), it should not be included as a synonym of *E. dubium*, which has 10 loculi, 16 testes and is found in *R. batis*. It is hoped that more work along the lines described above will contribute towards solving many of the difficulties regarding the systematics of the genus *Echeneibothrium*.

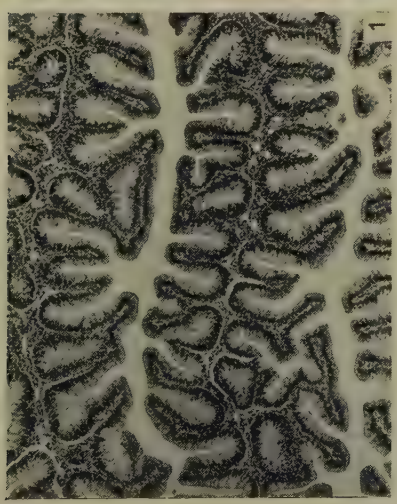
I am very much indebted to the late Prof. T. A. Stephenson, F.R.S., and Dr Gwendolen Rees for every possible encouragement to commence the above work with the aid of a Department of Scientific and Industrial Research Fellowship. My thanks are also due to the Director and staff, in particular Mr J. Green and Mr A. Mattacola of the Plymouth Marine Laboratory, and to Prof. James Brough for providing excellent research facilities. Dr J. Llewellyn, of the University of Birmingham, has kindly read the manuscript and suggested many improvements. Mr W. O'Grady has given technical assistance for which I am most grateful.

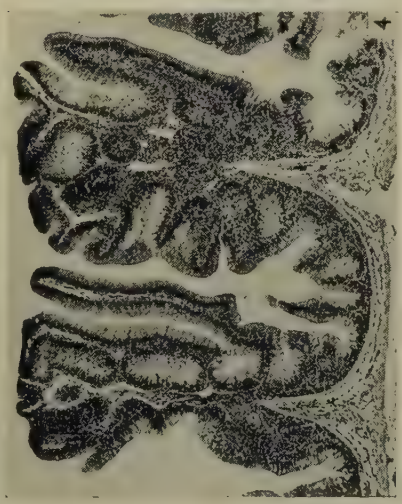
EXPLANATION OF PLATE I

Raia montagui. Facial and vertical sections through the mucosa from various regions of the intestine, all magnified to the same scale.

Fig. 1. First chamber.
Fig. 2. Third chamber where *Echeneibothrium maculatum* is usually attached.
Fig. 3. Last chamber.
Fig. 4. First chamber.
Fig. 5. Third chamber.
Fig. 6. Last chamber.







(*Facing p.* 650)

SUMMARY

Echeneibothrium maculatum has been studied in detail with particular reference to its ecology and anatomy. The species is characterized by the possession of 18–20 loculi in each bothridium, 20 testes in each proglottid and by its specificity to the anterior region of the intestine of *Raia montagui* only. It is thought that physiological, ecological and morphological factors may contribute towards this host-specificity. The taxonomy of the genus *Echeneibothrium* is discussed in the light of results obtained from a study of *E. maculatum*.

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STUDIES ON THE BIOLOGY OF LIMPETS

IV. THE BREEDING OF *PATELLA DEPRESSA* PENNANT ON THE NORTH CORNISH COAST

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(Text-figs. 1 and 2)

Patella depressa Pennant (= *P. intermedia* Jeffreys) has a very limited distribution in Britain. It is essentially a southern form, and is present along the south and west coasts from Bembridge, Isle of Wight, to Carmel Head, Anglesey, but has not been found in Ireland (Fischer-Piette, 1936; Crisp & Knight-Jones, 1955; Southward & Crisp, 1954; Crisp & Southward, 1958; unpublished records J. H. Orton and A. J. Southward).

On the shore *Patella depressa* has a restricted vertical range compared with the common limpet, *P. vulgata* L., and in south-west England is usually most abundant from MHWN to MTL: rarely is it found as high as MHWS or below MLWN (Fischer-Piette, 1938; Southward & Orton, 1954). It is not found in places sheltered from wave action (Evans, 1947*a*, *b*), and its preference for wave-beaten sites was amply confirmed by our quantitative observations on the Breakwater at Plymouth (Southward & Orton, 1954). However, it cannot apparently withstand severe wave action: at Shipman Head at Bryher, Isles of Scilly, and on the Eddystone Reefs, 10 miles SSW of Plymouth, it is replaced at all levels by *P. aspera*, a species which is usually restricted to low-water levels in more normal habitats.

From this brief analysis of the ecological niche occupied by *P. depressa* it is obvious that, although the species is widely distributed in the south-west, it was not easy to find places from which large samples could be taken at regular intervals. Favourable sites may be quite isolated and rough seas often prevent sampling. Furthermore, the species is difficult to distinguish by shell form, and experienced collectors are required. For these reasons sampling was concentrated at Trevone, north Cornwall, where reasonable samples can be obtained within easy walking distance, and the presence of outlying reefs gives some shelter at low water during heavy seas.

Most samples came from a level which provided the greatest numbers of large individuals, estimated as MTL to MLWN, near the seaward edge of the extensive reefs on the south-west side of Trevone Beach. There is a sewer outfall at the extreme seaward edge of these reefs, but its influence does not apparently extend to the site of limpet collection.

The samples were measured for shell length only, and then the sex and gonad stage assessed by cutting the foot from its attachment to the shell and turning it forward. The exposed gonad was graded according to the series of arbitrary stages selected for *P. vulgata* (Orton, Southward & Dodd, 1956). The larger specimens, from 31–45 mm shell length, were used for analysing the breeding sample, but smaller sizes were examined on several occasions to determine sex-proportions.

Some of the samples were sent by post from Trevone, to Liverpool and various marine laboratories where J.H.O. was working, and several days often elapsed before the sample was examined. An additional difficulty with *P. depressa*, not applicable to the other species investigated, is the smaller size of the mature animal, and hence of the gonad. There was thus a much greater possibility of confusion of stages, especially between developing and spawning stages of the same volume, which were separated in a more subjective way than the corresponding stages of *P. vulgata* (Orton *et al.*, 1956). Spawning males were recognized by the purplish tinge of the gonad, while spawning females could be distinguished only by the looseness of the eggs in the ovary.

The field-work was supported by grants from the Royal Society, and further assistance was provided by the University of Liverpool. Acknowledgements are due to Mr C. Roberts of Trevone for field collecting; to Dr D. W. Tucker, C. Grainge and the late P. Bond for help in collecting and examination of samples; and to Prof. J. M. Dodd for helpful criticism of the manuscript. Dr W. M. Lindley of Trevone kindly supplied invaluable local meteorological records.

SEX PROPORTIONS

The proportion of sexes found in the various size-groups of *Patella depressa* was determined on five occasions. The results are shown graphically in Fig. 1 as percentages of neuters, males and females in each 5 mm shell-length group. The samples were smaller than those used for determining the sex proportions in *P. vulgata*, since it was not easy to collect the ideal sample of 100 specimens in each size-group. From the figure it can be seen that complete differentiation of the gonads in the population is reached at approximately the same length as in *P. vulgata*, about 20–25 mm. Since the maximum length found rarely exceeds 45 mm, this means that a much larger proportion of the population of *P. depressa* is sexually inactive. Moreover, after about 35 mm an increasing proportion of secondarily neuter individuals is found, since the incidence of trematode infection, which can cause complete dedifferentiation of the gonad, is higher than in the common limpet. Development of both male and female gonads occurs at a much smaller size than in either *P. vulgata* or *P. aspera*, and 10–40% differentiated individuals were found in the 6–10 mm group. In all five samples the apparent 'development' of the males

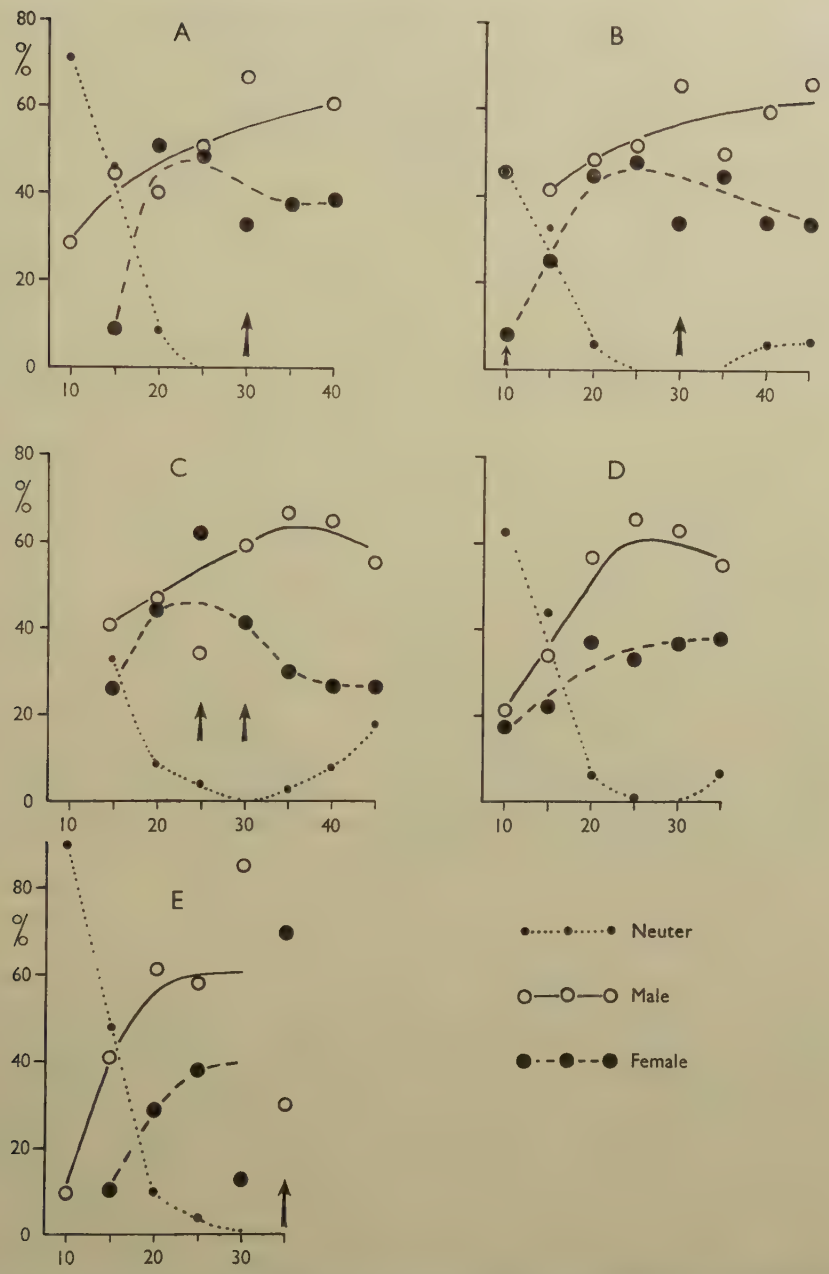

Fig. 1. Proportion of sexes (as percentage) in each 5 mm shell-length group of *P. depressa*, MTL–LWN, Trevone. A, 334 individuals, June 1946; B, 509 individuals, July 1946; C, 596 individuals, August 1946; D, 341 individuals, August 1948; E, 251 individuals, September 1948. It has been assumed that irregularities were introduced by the smallness of the samples, especially those marked with an arrow, and smooth curves have been fitted to the points by eye estimation.

appeared to be fairly steady, the proportion reaching a maximum of about two-thirds of the specimens of 25–35 mm and continuing at approximately the same level after allowance is made for the effect of parasitization. In the 1948 samples the proportion of females also increased fairly steadily with length, reaching maximum proportions of about one-third at 25 mm. These samples were taken more consistently from a particular habitat than the 1946 samples, which show a much more irregular 'development' of the females. From the 1946 samples it would appear that at 25 mm half the size-group were female, and that thereafter the proportion declined to the one-third level found among larger specimens in the 1948 samples. Mr C. Roberts, who collected most of the material, is sure that the 1946 samples came from the western edge of the site, which is subject to greater wave action than the more eastern parts from which samples were taken in later years, and may well have had a different rate of growth. It is also possible that a wider range of tide levels was sampled in 1946. Nevertheless, the agreement between the sets of samples in each of the 3 months is surprising, and it may be that there had been a more rapid development of young females during the preceding season, and a slower development of males, than in 1947–48.

All the samples examined for sex-proportions agree in showing the presence of males and females simultaneously in nearly all size-groups, with little significant variation in proportions after the disappearance of true undifferentiated specimens at 25 mm length. This trend offers great contrast with that in *P. vulgata* where over 90% males are present in the smaller size-groups, and a significant proportion of females begins to occur only in size-groups from which all neuter specimens have disappeared (Orton, 1946; Orton *et al.*, 1956). There is thus little prospect that protandrous hermaphroditism could occur in *P. depressa* in south-west England.

THE BREEDING CYCLE

Routine analysis of the samples of larger individuals was carried out as for *Patella vulgata*, and the results are presented in the same form, in Table 1 and Fig. 2, excluding parasitized neuter specimens. Fig. 2 also shows the mean air temperature (9 a.m. G.M.T. on standard screen) at Trevone for the first and second halves of each month. No sea-water temperatures were taken, and we have been unable to obtain any readings of sea-temperature for the period from neighbouring climatic stations. However, air temperatures, especially during the summer, have a considerable influence on intertidal animals (Southward, 1958), and when observed at the coast may have a similar trend to inshore sea temperatures.

From Fig. 2 it is obvious that *P. depressa* is a summer-breeding species in south-west England. In all 4 years redevelopment of the gonads began in April and May as temperatures rose, and was usually completed by June or

July. Spawning, as indicated by a rapid fall in the mean state of development of the gonad to below 1·0 and an increase in recognizable post-spawning and spent stages to 80% or more of the sample, occurred between the latter half of July and the first week in September. In each year it coincided with what we may term 'high summer'—the attainment of maximum air temperatures. During the following autumn and winter the proportion of recognizable

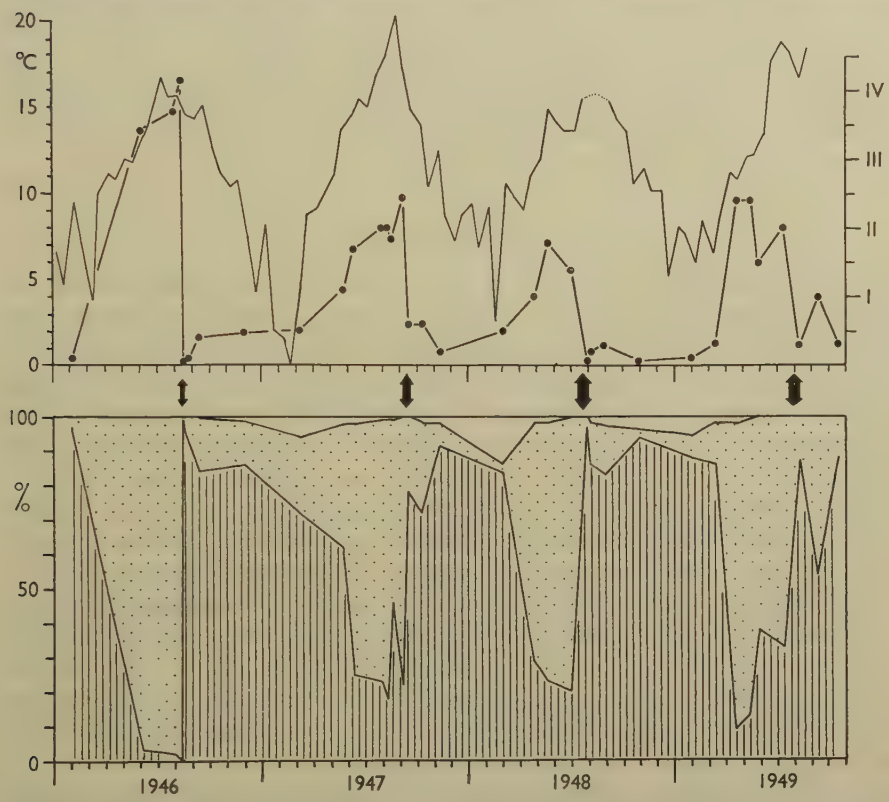

Fig. 2. The breeding cycle in *P. depressa*, MTL–LWN, Trevone. Lower graph: percentage of neuter, developing and post-spawning (including spent) stages in samples of individuals 31–45 mm shell length. Upper graph: mean state of development of the gonad (heavy line and circles), and twice-monthly mean air temperatures at Trevone (thin line). □, Neuter; ▤, developing; ▥, post-spawning & spent. Heavy arrows indicate the assumed spawning periods.

post-spawning and spent stages continued at a level of 70–80%, and although in some years (not 1948–49) some slight redevelopment of the gonad occurred soon after spawning, very few neuter stages were found until the early spring. Even then the proportion of neuter individuals remained below 15%, and there was no extensive resting condition for the population such as was found in *P. vulgata* at most British localities during the same years.

If details of the cycle are studied, we find considerable differences between the years. Some of these differences are undoubtedly due to sampling bias, especially in 1946 (see p. 656), and to the smallness of the samples in the last 2 years. The variations in the gonad index (MSD) in August 1947, May 1958, and May 1949 were probably due to inclusion of a few assessed post-spawning stages from a different habitat (e.g. pools) with a different rate of development of the gonad. However, there is also the chance that the period of spawning was less clearly defined, or that some slight spawning and redevelopment took place earlier in 1947 and 1949. These years were considerably warmer than the other 2 years studied, and a southern form may well be expected to respond to higher temperatures during its breeding period. The evidence is too incomplete for any definite explanation to be offered.

SPAWNING STIMULI

It is clear from Table 1 and Fig. 2 that in *Patella depressa* practically all the population begins spawning at the same time and that there is a fairly rapid voiding of gametes from the individuals. The process is well illustrated by the closely spaced samples of 1946. In that year 99% of the population began spawning between 12 and 14 August, and most of the individuals lost at least two-thirds of their gonad volume in those 2 days. The samples were taken further apart in 1947, but in September that year at least half the population began spawning between the 5th and 15th, losing two-thirds of their volume between those dates. Sampling was not carried out so frequently in 1948 and 1949, but there is no reason to suppose that spawning was less rapid.

In considering the breeding of *P. vulgata* it was shown that spawning might be stimulated by some external factor connected with the mechanical effects of rough seas on the shore. The relative sharpness of the spawning in *P. depressa* indicates that any spawning stimulus must be equally sharply defined. Air temperature can surely be ruled out as a possible stimulus, for although the peak of spawning coincided with or occurred just after the maximum temperature of the summer, the actual maximum varied from year to year by as much as 3°–4° C. The annual cycle of the species may well be keyed to the temperature cycle, so that the peak of gonad development occurs at the highest temperatures, which might be expected to be beneficial to a southern species at its northern limit (cf. Orton, 1920), but this is not a spawning stimulus.

Some difficulty arises when examining meteorological records (Air Ministry, 1946–49) for a possible connexion between the onset of spawning in *P. depressa* and strong winds. The site at Trevone is on a coast facing north-west and therefore sheltered to some extent from the S. and S.E. winds which are often strong in south-west England. On the other hand, the physical nature of the north Cornish coast is such that indirect swell can set up a heavy surf.

In the absence of observations on the state of sea it is not possible to be very precise as to the effects of various winds, but we might consider the sector from S.W. via N. to N.E. as being most likely to cause wave action.

In 1946, spawning at Trevone coincided with prevalent local storms, with S.W. winds up to gale force on 12 and 13 August, and there is little doubt that considerable wave action was present on the beach. In 1947 the peak of spawning was relatively late. This was a well-remembered summer in the south-west and winds of more than force 3 or 4 did not occur for some weeks before spawning. At St Eval, near Trevone, south-westerlies of force 5 were recorded on 9 September, and again on the 10th, 14th and 15th, while W.S.W. winds up to force 6 occurred on the 16th, just before the sample was

TABLE 1. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA DEPRESSA* FROM TREVONE

		Males										Females								
		Developing					Post-spawning					Developing					Post-spawning			
Date	No. in sample	I	II	III	IV	V	IV	III	II	I	N.*	I	II	III	IV	V	IV	III	II	I
1946																				
5 Feb.	31	—	—	—	1	—	—	3	6	8	—	—	—	—	—	—	—	—	4	9
6 June	61	—	2	15	14	4	—	1	1	—	—	—	2	10	12	—	—	—	—	—
29 July	95	—	—	7	49	5	—	1	—	—	—	—	7	6	20	—	—	—	—	1
12 Aug.	101	—	—	10	20	27	—	—	—	—	—	—	1	11	20	12	—	—	—	—
14 Aug.	107	—	—	2	—	—	—	9	59	11	—	—	—	—	—	—	—	—	13	13
22 Aug.	92	—	—	1	1	—	1	19	34	—	3	—	—	—	—	—	—	—	22	11
13 Sept.	110	—	6	7	1	—	—	9	58	3	—	—	1	3	—	—	—	—	16	8
2 Dec.	105	—	—	5	3	—	2	22	36	8	1	—	4	2	2	—	—	1	6	13
1947																				
7 Mar.	97	—	12	—	—	—	—	—	28	19	6	—	2	4	—	—	—	—	6	20
23 May	100	—	10	15	2	—	—	1	20	10	2	—	8	4	1	—	—	—	12	15
10 June	105	1	23	20	—	—	—	2	24	—	2	3	29	1	—	—	—	—	—	—
29 July	104	—	24	23	3	—	—	—	15	—	1	—	16	10	2	—	—	—	9	1
8 Aug.	108	—	25	33	2	—	—	2	11	1	1	—	13	12	2	—	—	—	4	2
19 Aug.	104	—	5	10	13	4	—	15	23	1	1	—	5	10	7	1	—	1	8	—
5 Sept.	109	—	6	24	10	—	—	12	8	—	—	—	—	10	21	7	8	—	1	2
16 Sept.	103	—	5	5	—	—	—	5	49	8	—	—	8	4	1	—	—	—	16	2
8 Oct.	98	—	15	6	—	—	—	2	27	11	2	—	4	—	—	—	—	—	18	13
8 Nov.	114	—	4	1	1	—	—	3	31	34	2	—	1	1	—	—	—	1	10	25
1948																				
1 Mar.	49	—	1	—	—	—	—	—	4	24	7	—	—	—	—	—	—	—	—	13
24 Apr.	51	8	11	3	—	—	—	—	—	10	1	11	2	—	—	—	—	—	—	5
19 May	56	2	22	8	—	—	—	—	1	—	1	2	7	1	—	—	—	—	8	4
29 June	60	13	18	—	—	—	—	—	1	9	—	12	5	—	—	—	—	—	—	2
28 July	34	1	—	—	—	—	—	—	—	26	—	—	—	—	—	—	—	—	—	7
3 Aug.	57	5	2	—	—	—	—	—	5	23	1	—	—	—	—	—	—	—	—	21
30 Aug.	57	—	5	—	—	—	—	—	23	8	2	1	2	—	—	—	—	—	8	8
29 Oct.	51	—	1	—	—	—	—	—	2	23	2	—	—	—	—	—	—	—	—	23
1949																				
31 Jan.	49	—	—	—	—	—	—	—	1	24	3	—	—	—	—	—	—	—	—	21
14 Mar.	55	—	3	2	—	—	—	—	7	21	1	—	2	—	—	—	—	—	—	19
21 Apr.	54	2	8	19	3	—	—	—	—	2	1	2	7	4	2	—	—	—	—	4
11 May	56	—	15	14	4	—	—	—	—	—	—	—	7	7	1	—	—	—	6	2
27 May	53	—	13	12	3	—	—	3	2	1	—	4	6	1	—	—	—	—	8	2
11 July	56	—	6	14	4	3	—	3	6	—	—	—	2	4	4	—	—	1	7	1
9 Aug.	54	—	6	—	—	—	—	—	25	8	—	—	1	—	—	—	—	—	5	9
10 Sept.	53	—	13	3	—	—	—	—	16	5	—	3	5	—	—	—	—	—	3	5
19 Oct.	55	1	5	—	—	—	—	—	5	24	—	—	—	1	—	—	—	—	3	16

* N. = Neuter

collected. During the presumed spawning period in 1948, from 29 June to 28 July, winds of at least force 5 or 6 were recorded on 6 days from directions between W.S.W. and N.N.E. In 1949 spawning occurred between 11 July and 9 August; W.S.W. winds of force 6 occurred on 2 days (1 and 2 August) during this period.

From this evidence it seems that there might be a relationship between wave action on the shore and the onset of spawning in *P. depressa*, but that further observations would be needed to show the relation closely. The stimulus required by *P. depressa* must be less intense than that needed by *P. vulgata*, since it spawns during summer storms of force 6 or so, while the latter species is exposed to equinoctial gales of force 8 to 9.

DISCUSSION

Reference has already been made to one major difference in the breeding cycle of *Patella depressa* compared with that of the common limpet: the absence of a resting stage when the majority of adult specimens have very reduced gonads of indeterminate sex. In *P. vulgata* this resting stage marks the nadir of breeding activity in spring and early summer. At certain Scottish localities sampled for *P. vulgata*, and especially at St Andrews (Orton *et al.*, 1956, table 5), the resting period was shorter, and the developing and post-spawning stages longer than at the more southern localities. It was suggested that there was a generally slower progression of the breeding cycle in the north, probably related to the lower temperatures experienced by the animals. This argument might be applied to the cycle in *P. depressa* at Trevone, where the species is not far from its extreme northern limit of distribution; a longer resting period, and quicker progression of the breeding cycle, may be found further south. It must be pointed out, however, that while the period of development is most prolonged in *P. vulgata* at St Andrews, it is the post-spawning and spent stages that are the longer in *P. depressa* at Trevone. In the latter case it is clear that the gonads cannot start redevelopment until the temperatures rise in the spring, and thus the period of development cannot be increased any further.

The most obvious differences between *P. depressa* and *P. vulgata* are in the period of spawning and the seasonal trend of the whole breeding cycle. At Trevone, during the 4 years studied, the gonads of *P. vulgata* did not begin to redevelop until July (sometimes as late as August), and the peak spawning took place in late October or November. The corresponding events in the cycle of *P. depressa* occurred some 4 months earlier and under very different climatic conditions. There is thus no overlap in the spawning periods of these species, and the chances of hybridization in nature are very remote.

The differences in breeding behaviour reinforce the already established differences in morphology, habitat and distribution, and serve to emphasize

the real nature of the species concerned. We can no longer regard with any satisfaction the concept that *P. vulgata* and *P. depressa*, as well as other species of *Patella*, are ecological or distributional variants of one super-species (cf. Fischer-Piette, 1935, 1938).

SUMMARY

The breeding of *Patella depressa* was studied at Trevone, North Cornwall, for 4 years. Gonads were examined and assessed by methods described in a previous report on *P. vulgata*.

In contrast to the common limpet, *P. depressa* is a summer breeder in Cornwall. Gonad development begins in the spring and is complete by June or July. Spawning coincides with maximum air temperatures in July, August or early September. The autumn and winter period is passed with gonads in recognizable post-spawning and spent stages, and only a small proportion of completely resting neuter gonads is found in the early spring.

Mass spawning takes place, and gametes are voided rapidly. No definite spawning stimulus can be shown, but, as with *P. vulgata*, there may be some relation between onset of spawning and the occurrence of wave action on the shore.

The differences in breeding behaviour of *P. depressa* and *P. vulgata* reinforce the other differences between them and throw some doubt on the concept of super-species in *Patella*.

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BIOLOGICAL DIFFERENCES BETWEEN SEA WATERS: EXPERIMENTS IN 1960

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(Plates I–III)

The years which have elapsed since we first published on this subject (Wilson, 1951) have seen much confirmation that, in the laboratory, the development of the sea-urchin (*Echinus esculentus*) and of other species is influenced by some unknown property or properties of sea water, which varies from one locality to another and in the same locality from time to time, and can vary with depth at the same station. The property would appear to be linked with the distribution and movements of oceanic and coastal water masses, but of what it consists we have as yet no certain knowledge. We have tested various hypotheses, our own and others propounded to us, but so far every such test has been negative. Recently Bougis (1959) has suggested that copper concentration in sea water, which is known to be variable in distribution and in time, could explain our results. He supported his hypothesis with careful experiments in which he reared larvae of *Paracentrotus lividus* in sea water to which copper sulphate was added to increase the copper concentration to desired amounts. He found that with increasing copper concentration the size range of arm-spicule length was reduced. A significant reduction was obtained with concentrations equivalent to those known to occur in natural sea water; the reduction was evident at 5 μg/l. Cu, and it was well marked at 10 μg/l. Cu and above. Bougis quoted figures for copper concentrations from several parts of the world giving ranges from 1 to 34 μg/l. Cu. In particular he quoted figures from Atkins (1953) showing an annual variation for surface water at station E 1 from 1·5 μg/l. Cu in autumn to 24·8 μg/l. Cu in winter. Other figures from Atkins were quoted, including a contrasting pair for E 1 on 13 April 1949 when the surface water showed 8·1 μg/l. Cu and the deep water at 50 m showed 18·3 μg/l. Cu. He rightly pointed out that the copper concentrations for the waters we used had never been determined. He wrote and asked if it were possible for us to verify his hypothesis, and in view of his work this seemed a very reasonable request.

Earlier than Bougis, Bernhard (1957) had shown similar effects of copper on the development of *Arbacia lixula* larvae and he had published photographs showing a great difference between *Arbacia* larvae reared in water from the Gulf of Naples and in the same water after chelation. In the latter the larvae were normal, in the former abnormal.

Our present series of experiments were thus primarily designed to test Bougis's hypothesis. It was necessary to do them in the manner of our earlier experiments, using waters from contrasted localities, and not merely to repeat Bougis's and Bernhard's methods in which only natural sea water from one locality had been used.

COLLECTION OF MATERIALS

There was a scarcity of *Echinus esculentus* on the trawling grounds in 1960, attributed mainly to the activities of French scallop-dredgers working extensively over the grounds for months previously. Sea-urchins with greenish patches were often trawled up—an indication that they had been damaged some days earlier, probably dredged up and thrown back into the sea. The best urchins were got by underwater swimmers from rocky grounds inaccessible to scallop-dredgers.

An attempt to find plankton-rich water to the westward failed[1] and we therefore chose to use water from the Firth of Clyde as a probable good water to contrast with a probable bad water from E 1. In the past Clyde water has often proved to be a better medium for developing eggs and larvae of sea-urchins, and of some polychaetes, than water from the Plymouth district. We extend our thanks to the Millport Marine Station and to Mr E. Latham for sending us water from the Firth of Clyde.

GENERAL METHODS

Our methods have been described in detail in earlier papers (Wilson, 1951; Wilson & Armstrong, 1952, 1954, 1958) and there was no radical departure in the present experiments. As usual all water tested had first been passed through sterilized Doulton filter candles. We have standardized on three 175 ml. Pyrex glass crystallizing dishes for each test set, and we put 100 ml. of the water to be tested into each. The fertilized eggs, after washing in the manner described for each experiment, are distributed to the dishes with a special glass ladle of capacity 8 ml., and this volume of wash water with eggs is added to the 100 ml. already in each dish. Unless otherwise stated, the larvae of all three dishes in any one test set were identical in appearance and behaviour. Also, unless otherwise stated, swimming larvae were not decanted into clean empty dishes to separate them from undeveloped eggs or dead larvae. Throughout each experiment all dishes were examined with a hand-lens once or twice a day, and the contents of at least one dish of each test set, usually all three, were studied more closely on the stage of a dissecting binocular. A wide-mouthed pipette was used for removing larvae for preservation, or they were merely poured off into another dish and quickly flooded with near-absolute alcohol. After hardening, and before mounting, these preserved larvae were taken down in gradual steps to distilled water to remove precipitated salts, and then up again through a range of alcohols to be mounted without staining in Euparal. All alcohols and the distilled water were rendered alkaline with traces of borax.

[1] Expt. 1 includes a test of a water collected on this cruise; the result confirms our suspicion that this Western water was poor (see p. 669).

CHEMICAL WORK

Copper determinations and adjustment

For Expts. 1 and 2 a biquinolyl method (Riley & Sinhaseni, 1958) was chosen because it is specific and has been particularly well tested. It is likely that the results give the total copper content of the water to the nearest 0·2 μg Cu/l. Copper determinations had not originally been planned for Expt. 3, so, since time was short, the easier and quicker diethyldithiocarbamate method was used. The very straightforward version given by Barnes (1959, pp. 229–30) was employed. The results are believed to be reliable. Copper concentrations found are given in the Table below, which includes some values for water before and after filtration through porcelain candles. The effect of this filtration in reducing the copper content of the samples is clear; the E 1, Clyde and outside waters have lost respectively 75 %, 30 % and 60 %.

TABLE A

Date of analysis	Method	Sample	Copper content
19. iii. 60	Biquinolyl	E 1, 9. iii. 60, filtered	0·6
		Clyde, 9. iii. 60, filtered	1·5
		Western, 9. iii. 60, filtered	0·8
14. iv. 60	Biquinolyl	E 1, 4. iv. 60, unfiltered	2·0*
		E 1, 4. iv. 60, filtered	0·5*
26. iv. 60	Diethyldithio-carbamate	Outside, 20. iv. 60, unfiltered	1·3
		Outside, 20. iv. 60, filtered	0·9
		Clyde, 21. iv. 60, unfiltered	4·2
		Clyde, 21. iv. 60, filtered	1·7

* Not used for the *Echinus* experiments.

Adjustment of copper contents to the levels required in the experiments was made by adding small volumes of a standard solution of cupric sulphate (copper content 5 μg/ml.) to 500 ml. portions of sea water, using a microburette.

Chelated samples

An approximately 0·1 M solution of the complexing agent (E.D.T.A.) in sea water was first made by dissolving 18·7 g of the disodium salt of ethylene-diamine-tetra-acetic acid in 500 ml. of a mixture of E 1 and Clyde waters, and then adding solid sodium hydroxide to bring the hydrogen-ion concentration to pH 9·09.

Sea-water samples with approximately 0·001 M E.D.T.A. were made as follows:

Mixed water, chelated

2 l. E 1 + 2 l. Clyde (pH of mixture 8·11) + 40 ml. 0·1 M E.D.T.A. in sea water + 5 ml. 0·1 N-NaOH. pH after aeration 8·03.

E 1, *chelated*

1 l. E 1 (pH 8·12) + 10 ml. 0·1 M E.D.T.A. in sea water + 2 ml. 0·1 N-NaOH. pH after aeration 8·08.

Clyde, chelated

1 l. Clyde (pH 8·11) + 10 ml. 0·1 M E.D.T.A. in sea water + 2 ml. 0·1 N-NaOH. pH after aeration 8·09.

Volatile matter

The method described below was intended to provide sea-water samples free from, and enriched in, the recently reported volatile fraction (Armstrong & Boalch, 1960). Lucas (1955) has suggested that the results of some of our heat-treatment experiments (Wilson & Armstrong, 1954, pp. 350 and 359) may be due to the existence of a beneficial substance of a more or less volatile nature.

2·5 l. of sea water were distilled in an all-glass apparatus comprising a 3 l. boiling flask, 50 cm fractionating column packed with Raschig rings, splash trap, condenser, ice trap and 250 ml. receiver. The first 250 ml. of distillate containing most of the volatile matter was kept in a stoppered flask. A second 250 ml. portion was discarded. Loss of carbon dioxide during the distillation caused the sea water to become cloudy with a heavy precipitate of calcium carbonate, and after the distillation a stream of carbon dioxide was passed into the hot turbid liquid. The calcium carbonate dissolved, leaving a clear solution in less than 1 min. The flask and contents were rapidly cooled, and excess carbon dioxide removed by aspirating with clean air. The volume was then adjusted to 2·0 l. with distilled water. The mixtures shown in Table B were made for the experiments.

If most of the volatile matter is assumed to have been collected in the first 250 ml. of distillate, then the enriched samples have double the normal concentration, and the original salinities are preserved unchanged. It must not be forgotten that boiling the samples may affect their characteristics. Since the initial acidities of those made with volatile fractions was high because of excess of carbon dioxide, the precaution was taken of allowing the 100 ml. portions in the experimental dishes to stand, partly uncovered, in order to approach equilibrium with the atmosphere. Any other method of reducing the acidity might have introduced undesirable complications. A separate set of dishes was kept for pH determinations, the results of which are as follows:

TABLE B

400 ml. concentrated sea water + 100 ml.	pH on mixing	pH after standing (not larvae dishes)			
		1 h	2 h	4 h	Overnight
E1 + double distilled water	7·92	—	—	—	—
E1 + E1 volatile fraction	6·69	6·85	7·08	7·04	7·88
E1 + Clyde volatile fraction	6·79	6·92	7·02	7·11	7·80
Clyde + double distilled water	7·89	—	—	—	—
Clyde + E1 volatile fraction	6·62	6·81	7·00	6·98	7·84
Clyde + Clyde volatile fraction	6·59	6·97	7·10	7·19	7·83

THE EXPERIMENTS

The experiments were carried out in a room on the north side of the building. It was not possible to control room temperature, which was recorded with a thermograph. The range was never more than a few degrees Centigrade.

EXPERIMENT 1

E1 water collected 9. iii. 60. Drawn up from 15–20 m below surface through a polythene tube; water not passed through pump; strained through sterilized 200-mesh bolting silk into specially cleaned carboy.

Ship: R.V. 'Sarsia' with F.A.J.A.

Salinity: 35·43‰. pH 8·11. Copper content after filtration 0·6 μg/l.

Western water collected 11. iii. 60. Method of collection as for E1 water.

Position: 50° 00′ N., 7° 00′ W.

Ship: R.V. 'Sarsia' with F.A.J.A.
Salinity: 35·35‰. pH 8·08. Copper content after filtration 0·8 μg/l.
Clyde water collected 9. iii. 60, 13.30 h. Water dipped from surface with polythene bucket and strained through polythene funnel and 200-mesh bolting silk (sterilized in Plymouth) into specially cleaned carboy.
Position: mid-Largs Channel, ¾-ebb, wind S.E. force 4.
Boat: R.V. 'Mizpah'.
Salinity: 32·03‰. pH 8·10. Copper content after filtration 1·5 μg/l.

The waters to be tested, in their untreated and treated forms, are listed in Table 1.

The fertilization was made at 6.15 p.m. on 22 March 1960, using one female and one male selected from a quantity of recently collected sea-urchins. Each was caused to spawn first into a mixture of Clyde and E 1 waters in equal proportions, and then into a similar mixture of the same waters chelated.

TABLE 1. EXPERIMENT 1

For Sets IV–VI the fertilization was made in Mixed water chelated; for all other Sets in untreated Mixed water (50% Clyde:50% E 1).

Set		Eggs washed in
I	Clyde water, Cu, 1·5 μg/l.	Clyde water
II	E 1 water, Cu, 0·6 μg/l.	E 1 water
III	Mixture, 50% Clyde, 50% E 1 water	Mixed water
IV	Clyde water, chelated	Clyde water, chelated
V	E 1 water, chelated	E 1 water, chelated
VI	Mixture, proportions as Set III, chelated	Mixed water, chelated
VII	Clyde water, Cu increased to 10 μg/l.	Clyde water
VIII	Clyde water, Cu increased to 20 μg/l.	Clyde water
IX	Clyde water, Cu increased to 30 μg/l.	Clyde water
X	E 1 water, Cu increased to 10 μg/l.	E 1 water
XI	E 1 water, Cu increased to 20 μg/l.	E 1 water
XII	E 1 water, Cu increased to 30 μg/l.	E 1 water
XIII	E 1 water, Cu increased to 1·5 μg/l.	E 1 water
XIV	Western water, Cu, 0·8 μg/l.	Mixed Clyde and E 1 waters

The fertilized eggs gave well erected fertilization membranes; a very small proportion of the eggs did not fertilize. The two similar sized batches of eggs were each divided, shortly after fertilization, into three equal portions, making six portions in all. During the next hour and a half each portion was washed in six changes of a selected water so that at the end of that period there were eggs in Clyde water, E 1 water, and the two waters mixed, and also in the same waters chelated. From these washed eggs the test dishes were supplied shortly after first cleavage; Table 1 records from which wash waters the eggs in each set came. Each dish received approximately the same number of eggs, which spread thinly over the bottom. Cleavage continued in a normal manner for all eggs except a small proportion which divided irregularly.

Blastulae swam up in all dishes between 2 and 3 p.m. the next day. They swam up higher and in greater numbers in Clyde waters (all treatments) than in equivalent E 1 waters, and in unchelated waters than in equivalent chelated.

In the mixed water of Set III the blastulae were the close equals of those in Set I (Clyde water), swimming more strongly than the blastulae of Set II (E 1 water). In the chelated Sets IV–VI the larvae in the Clyde water and the mixed water were again about equal and were a little more vigorous than those in the E 1 water, though the distinction was less clear than in the unchelated waters. With increased copper concentration (Sets VII–IX and X–XII) the number of blastulae swimming decreased, being especially noticeable in Sets X–XII. However, in Set XIII (E 1 water to which there had been a slight addition of copper to bring the concentration equal with that in the Clyde water) swimming vigour was improved over Set II (E 1 water) and was the near equal of that in Set I (Clyde water). The blastulae in the Western Water of Set XIV swam a little feebly, as did those in the E 1 water of Set II. Structurally the blastulae of all sets looked alike.

Throughout the following day, 24 March 1960, when the larvae were gastrulae, the same relative distinctions between sets persisted. Some morphological differences now become apparent. The gastrulae in Set I (Clyde water) were a trifle larger and more transparent than those in Set II (E 1 water), while those in the chelated waters had a dwarfed appearance, especially so in Set V (E 1 water chelated) as compared with Set II (E 1 water unchelated). There was perceptible dwarfing in the highest copper concentrations (Sets IX and XII). Strikingly the larvae in Set XIII (E 1 water with a minute addition of copper) were a little better developed than those in Set II (E 1 water).

As development continued there was some loss of swimming vigour in all sets but the relative differences between sets remained as before. There were few or no deaths but some larvae developed faster or more normally than others; this was true in any dish from any set. While the dishes of any one set were all alike as regards larval content, the differences between the larvae, structurally and behaviourally, of one set and another became accentuated. These were well marked on 25 March 1960, but a better comparison can be made for the following day when the larvae were nearly 4 days old. During the morning of that day, from one dish of each set there were withdrawn with a pipette a fair sample of the larvae swimming in mid-water and from off the bottom; these were fixed in alcohol and subsequently mounted unstained in Euparal for study of their skeletons, these observations to be added to those made on the larvae while still alive. It is thought that these preserved samples may contain a rather undue proportion of the more poorly developed larvae from the bottoms of the dishes than of the better developed larvae swimming well up from the bottom. This should be remembered when comparing the photographs on Pl. I of some of these preserved larvae.

On this day, 26 March 1960, the larvae of Set I (Clyde water—Pl. I, fig. 1) were more uniform in size and structure than those in Set II (E 1 water—Pl. I, fig. 2) where there was greater variation in these respects with decidedly more shrunken abnormals. But in the E 1 water there were many normal and

near-normal four-armed plutei, with elongate bodies and slender post-oral arms. At the time of fixation the tissues of one or more of the arms of some of these plutei had begun to shrink down the skeletal rods, leaving the ends of the latter protruding. In the Clyde water the best of the larvae were somewhat squat with short and broad post-oral arms and scarcely distinguishable anterior lateral arms with no signs of such shrinkage. Moreover, while the plutei in the E 1 water had well-formed skeletal structures, even the most misshapen almost spherical larvae having some spicules, the best of the Clyde larvae had poorly developed skeletons, abnormally irregular and with elements missing. Such rods as were present were very thin; often there was no skeleton at all, or the barest traces, especially in the less well-formed larvae. The difference between the larvae of one water and those of the other were striking and unmistakable: morphologically there was a 'Clyde-type' of larva and an 'E 1-type', the latter in appearance approximating the more closely to what is usually figured as a 'normal' pluteus for this stage in development. No Clyde-type larva was ever found in E 1 water and no E 1-type in Clyde water. The distinction between a swarm of larvae reared in one kind of water and a similar swarm reared in the other was even clearer while they were alive than is to be appreciated from the photographs of a few preserved specimens. Only in the mixed waters was there a tendency for both types to be present, especially in the chelated mixed water (Set VI). In the unchelated mixed water (Set III) the E 1-type predominated. Chelation of the waters (Sets IV and V, Pl. I, figs. 3 and 4) had done nothing to improve the larvae; they were if anything slightly inferior to the larvae in the corresponding unchelated waters of Sets I and II.

It is the sets with added copper which are of most interest. Throughout the Clyde water series (Sets VII–IX) the larvae were all Clyde-type, with a proportion of almost spherical abnormals (Pl. I, fig. 5, is typical); while throughout the E 1 water series (Sets X–XII) the larvae were either E 1-type or showed varying degrees of imperfection down to shrunken spherical abnormals (Pl. 1, fig. 6, is typical). The higher copper concentrations adversely affected the larvae but the morphological type accorded with the kind of water used and not with the copper content.

Actually the copper content had little adverse effect on the larvae until it exceeded 10 μg/l., and even at 20 μg/l. it was not great. It was pronounced at 30 μg/l. The most unexpected result was the effect of the minute addition of copper to E 1 water in Set XIII (Pl. I, fig. 7). The plutei here were unmistakably better than any others in the whole experiment, being well developed E 1-types with longer arms, better skeletons and a smaller proportion of abnormals than in Set II containing the same E 1 water without added copper.

In the Western water (Set XIV—Pl. I, fig. 8) the plutei were all very poor and abnormal in some degree and somewhat different in appearance from

either E 1 or Clyde larvae, but it would not be profitable to attempt a detailed description.

The experiment was discontinued at the fourth day because towards the end of that day some larvae died and others lost their arms. Neither of the two main waters would appear to have been as 'good' as the best waters of some previous years, and perhaps neither was particularly 'bad'. They were unmistakably different and the basic difference was not masked by chelating or by the addition of copper. The fertilization, by all the usual indications such as well-raised fertilization membranes, seemed to be of good average quality and could have been expected to have given a high proportion of normal plutei, other conditions being favourable.

At the beginning of the experiment the room temperature was 18° C; it fell rapidly to 16° C and then more slowly during the next 12 h to 14° C. After that it varied between 14 and 16·5° C falling to 12·5° C right at the end.

EXPERIMENT 2

The waters used were the same as in Expt. 1, but owing to most of the stock of E 1 and Clyde waters having been used up in that experiment the fertilization had to be made in the Western water. One female and one male were selected from about two dozen freshly trawled sea-urchins which were opened for examination of the gonads. The shed ova were fertilized at 6.15 p.m. on 31 March 1960 and the well-raised fertilization membranes of almost all eggs indicated full maturity. After fertilization the eggs were washed twice in Western water and then divided into two equal portions, one of which was washed with four changes of Clyde water and the other with four changes of E 1 water.

The experiment was designed to test, first, the effect of small additions of copper to the E 1 water to check the result obtained in Expt. 1, Set XIII. In five triplicated tests the copper concentration was stepped up in equal intervals from 0·6 to 9·6 μg/l. Secondly, it was designed to test the effect of adding to the natural waters volatile fractions obtained from the E 1 and Clyde waters (see p. 666). Six tests including the controls, each test triplicated, were supplied in the usual way from either the Clyde or E 1 final wash-waters.

Although the fertilization in its early stages looked good, many eggs ceased development at first cleavage and many others divided irregularly or disintegrated. Only a very few blastulae of normal appearance were produced in any of the dishes. These swam up from the bottom about 21 h after fertilization. At 22 h two dishes of each set were decanted into clean dishes so that only these swimming blastulae and most of the water was saved. There were so few living larvae in any one dish, however, that comparisons between the various tests could scarcely be made and the most that can be recorded is that the small additions of copper indeed appeared to promote swimming vigour,

and that the volatile fractions were without noteworthy effect. By the third day so few larvae remained alive that this part of the experiment was abandoned.

We return to consider the larvae in the wash-beakers. As usual these had been kept as controls after supplying the test dishes with eggs. Each still contained a very large number of eggs and so it was that from them a fair number of blastulae rose to the surface. These were poured into clean beakers (A and B, Table 2) with some of the water and more clean Clyde or E 1 water added to each. By the third day, 3 April 1960, it had become perfectly clear that whilst in both beakers there was a wide range of structural form between the most normal larvae and the most deformed, the best of the larvae in the E 1 water had longer arms and were superior structurally to those in the Clyde water. The difference was of the same order as had been apparent in Expt. 1.

TABLE 2. PART OF EXPERIMENT 2

Larvae from beakers, fixed 11 a.m. 4. iv. 60 (nearly 4 days old).

	Beaker A, Clyde water	Beaker B, E 1 water
Number of mounted larvae counted and classified	30	103
Normal	33·3 %	50·4 %
Slightly abnormal	36·3 %	26·2 %
Very abnormal	30·3 %	23·3 %
Appearance of normal plutei	Squat rounded bodies with short post-oral arms and barely perceptible anterior lateral arms	Conical bodies with long post-oral arms and short anterior lateral arms
Condition of the calcareous skeleton	None	Often none, but some plutei with very slender rods, or parts of rods. A very few have well formed body rods

On 4 April 1960 the larvae were distinctly Clyde-type and E 1-type respectively (Pl. II, figs. A, B). Also the larvae were swimming more strongly in the E 1 water than in the Clyde where many had sunk to rest on the bottom. The swimming larvae of both vessels were poured off into clean beakers after some had been removed for preservation. Both lots were now divided equally to make four lots in all, two of Clyde water and larvae and two of E 1 water and larvae. To one beaker of each sort an equal volume of clean unused water of the same kind was added, to the other beaker an equal volume of clean unused water of the other kind. This gave the mixtures as set out in Table 3. It should be noted that as fewer larvae had been swimming in the Clyde water on this day than in the E 1 there were three or four times as many larvae in E 1-derived beakers (B 1 and B 2 in Table 3) than in the Clyde-derived beakers (A 1 and A 2).

Two mornings later, on 6 April 1960, there were no larvae swimming in beakers A 1 and A 2, hardly any in beaker B 1, but at least 20 in beaker B 2. However, as all on the bottoms of the beakers were alive, all four beakers

TABLE 3. PART OF EXPERIMENT 2

Larvae from beakers fixed 10.35 a.m., 7. iv. 60 (nearly 7 days old).

	Beaker A 1	Beaker A 2	Beaker B 1	Beaker B 2
Water larvae were in until 4. iv. 60	Clyde water	Clyde water	E 1 water	E 1 water
Equal part clean water added 4. iv. 60	Clyde water	E 1 water	E 1 water	Clyde water
Number of mounted larvae counted and classified	53	57	129	151
Normal	18·8 %	26·3 %	22·4 %	43·0 %
Slightly abnormal	13·2 %	26·3 %	45·7 %	39·0 %
Very abnormal	67·8 %	47·3 %	31·7 %	17·8 %
Appearance of the normal plutei	Arms of moderate length	The best have longer arms and are larger than any in Beaker A 1	Arms longer and plutei generally larger than in Beaker A 2	In general better developed plutei than in the other beakers
Condition of the calcareous skeleton	Usually none. Some specimens with very thin incomplete rods	Usually none but very thin rods a little more frequent than in Beaker A 1	Good skeletons in many plutei but rods sometimes very thin or partially lacking. Some very abnormal larvae are without spicules	Good skeletons as in Beaker B 1, if anything better. All the very abnormal larvae have at least some spicules
Arm shrinkage of the slightly abnormal plutei	In the absence of skeletal rods it is uncertain if any shrinkage has taken place	As in Beaker A 1	The high proportion of slightly abnormal larvae is due to so many otherwise normal plutei with shrinking tissues exposing arm rods	Relatively few plutei with shrinking arm tissues

were stirred up. In A 1 and A 2 all except one or two larvae quickly sank down again but in B 1 a considerable number swam for some time, while in B 2 between 30 and 40 were still swimming well up late in the evening.

The following day, 7 April 1960, there was no larva swimming above bottom in any beaker, though all were alive. It was observed that some of the plutei in beakers B 1 and B 2 had protruding arm rods; the living tissues were shrinking to expose them. Consequently all larvae were preserved at once. They were examined in alcohol immediately after preservation, and more thoroughly later after mounting. In Table 3 the main features of these larvae are compared; some of the preserved specimens are seen in Pl. II, figs. A 1, A 2, B 1 and B 2. There is revealed a series improving from the poorest larvae in beaker A 1 to the best and very much better larvae in beaker B 2. Again it is apparent that the E 1 water was superior to the water from the Clyde, but what is so striking is that the E 1 water was actually improved by the addition of Clyde water at a time when the larvae were 4 days old. The addition of E 1 water to Clyde water at the same larval age also affected improvement, though not to quite the same extent. This is the first time in the history of our experiments that a mixed water has proved superior to either of the components alone.

It will, quite rightly, be objected that these results were obtained with relatively few larvae, themselves the survivors of a fertilization in which the majority of the eggs died, and moreover that there was only one test beaker for each kind of water. But it should be remembered that it is normal embryological practice to pour off swimming larvae from moribund eggs and subsequently to rear them through pelagic development, and that in our experiments when several dishes of the same sort have stood side by side it has been rare for any one to vary to an appreciable extent. These considerations incline us to accept these results without at the present time being able to do more than guess at an interpretation.

At the beginning of the experiment the room temperature was 14° C; within the first 24 h it rose to 17° C and fell to 15° C, thereafter varying slowly between 16·5° C and 13° C.

EXPERIMENT 3

The results of the first two experiments made it desirable to test new samples of waters from E 1 and from the Firth of Clyde. Circumstances however prevented water being got nearer than some miles from the E 1 position. Particulars of the waters are as follows.

Outside water collected 20. iv. 60. Ship: M.V. 'Sula'.

Position 5 miles east from Eddystone, approx. 50° 11′ N., 4° 8′ W.

Collected with polythene bucket and strained through 200-mesh bolting silk into glass carboy, all specially cleaned beforehand.

Salinity: 35·06‰. pH 8·03. Copper: before filtration 1·3 μg/l., after filtration 0·9 μg/l.

Clyde water collected 21. iv. 60, 10.50 a.m., using longboat.

Position mid-Largs Channel, approx. 55° 45′ N., 4° 54′ W. Tide ¾-ebb, light W. wind. Collected with polythene bucket and strained through 200-mesh bolting-silk into glass carboy, all specially cleaned beforehand.

Salinity: 28·90‰. pH 8·17. Copper before filtration 4·2 μg/l., after filtration 1·7 μg/l. Although the water had been strained through bolting-silk there was a noticeable *Skeletonema* content in the water on arrival at Plymouth. This was removed by filtration.

Included in the experiment were tests of mixtures of the waters; tests of Outside water with copper added in minute amounts; and tests of both waters to which had been added volatile fractions obtained from them (see p. 666). The experiment was arranged as indicated in Table 4.

TABLE 4. EXPERIMENT 3

Fertilization made in Mixed water (50% Clyde: 50% Outside water).

Set		Eggs washed in
I	Clyde water	Clyde water
II	Outside water	Outside water
III	75% Clyde + 25% Outside	¾ of the eggs in Clyde; ¼ of the eggs in Outside
IV	50% Clyde + 50% Outside	½ of the eggs in Clyde; ½ of the eggs in Outside
V	25% Clyde + 75% Outside	¼ of the eggs in Clyde; ¾ of the eggs in Outside
VI–VIII	Outside water + Cu in quantities not exceeding 10 μg/l.	Outside water
IX–XI	Clyde concentrate with added volatile fractions or distilled water for control	Clyde water
XII–XIV	Outside concentrate with added volatile fractions or distilled water for control	Outside water

Subsidiary vessels

	(After decanting at 22 h)		
Beaker 1	(1*a*)	Clyde water	Clyde water
Beaker 2	(2*a*)	Outside water	Outside water
Beaker 3	(3*a*)	Mixed water (50% Clyde: 50% Outside)	½ of the eggs in Clyde water; ½ of the eggs in Outside water
Dishes X and Y		Mixed water (50% Clyde: 50% Outside)	Mixed water (50% Clyde: 50% Outside)

After 24 h the blastulae in dish X were divided and distributed into 2 dishes of Clyde water, 2 dishes of Outside water, 3 dishes of Mixed water (50% Clyde: 50% Outside).

Great difficulty was experienced in obtaining sea-urchins, several days of collecting at sea procured less than 40, many small. They were opened on 27 April and were all found to be spent, or to have ova undergoing cytolysis. Five large sea-urchins from the aquarium, where they had been for several months, were next opened; four were male, one female and all fully mature. The female and one male were caused to spawn on beakers, both ova and

sperms were shed freely and seemed to be in perfect condition. After fertilization almost all eggs had well-raised fertilization membranes and the early cleavage stages, as far as the eight-cell stage, were regular and normal.

The fertilization was made at 6.10 p.m., 27 April 1960, in a mixture of equal parts of the two waters; and it was into this same mixture that the urchins had been spawned. A dish (X) of this mixed water with eggs covering the bottom in a rather thick layer was set on one side, and a smaller dish (Y) with eggs in a thin layer was used to observe the development process during the first 3–4 h. A sufficient quantity of eggs was now divided equally between two beakers while still in the mixed water; as soon as this had been done one batch was washed in six changes of one kind of water, the other batch in six changes of the other water, all changes being completed before first cleavage. There followed the usual distribution to the test dishes, each receiving an equal quantity of eggs which scattered thinly over the bottom. Table 4 shows from which wash-water the various sets were supplied. The two wash-beakers were finally topped up with their respective kind of water and from them a third beaker was derived by mixing an equal quantity of water and eggs from both. These three beakers were to serve as additional controls.

At 10 a.m. the next day it was found that almost all the eggs in all vessels with undiluted Outside water (Sets II, VI-VIII, XII–XIV, beaker 2) were dead and closer examination showed that they had in the later cleavage stages divided irregularly before dying. But there were some early blastulae apparently normal. In vessels with undiluted Clyde water (Sets I, IX–XI, beaker 1) although there were fair numbers of living abnormal stages the majority were good-looking early blastulae beginning to move. In the mixed waters Sets III–V the proportion of abnormal stages was lower in Set III than in Set IV and highest in Set V, and was in rough accordance with the proportion of Outside water in the mixture. (It is important to note from Table 4 that the eggs were derived from the two wash waters in the same proportion.) In striking contrast dishes X and Y containing eggs which had been in mixed water continuously since fertilization had almost no abnormals; here nearly 100% of the eggs had developed into perfect blastulae. These swam up slowly and when 24 h old those in dish X were distributed equally between three new dishes of mixed water, two dishes of Clyde water and two dishes of Outside water (see foot of Table 4). About 2 h earlier blastulae swimming near the surface in the three beakers (nos. 1–3 in Table 4) had been poured off into a second set of clean beakers (nos. 1*a*–3*a* in Table 4) which were then partially filled with more water of the kind appropriate to each. It should be noted that fewer swimming blastulae were obtained from the original Outside water beaker than from either of the other two.

Because the death-rate in all dishes with Outside water was so high the tests with copper and with volatile fractions were inconclusive. They were also inconclusive for the tests with volatile fractions in the Clyde water

because, as will be described below, larvae in Clyde water alone did not develop normally. The dishes of these sets are therefore of interest only in that their contained eggs and larvae behaved according to the source of the water, as they did in other vessels with water to which no additional substances had been added. But the further development of the blastulae from dish X and those decanted from the beakers is of interest; the former will be considered first.

Some 16 h after transferring the blastulae from dish X to other dishes as described above, the swimming vigour of the gastrulae (which stage they had then reached) was markedly less in the two Outside water dishes than in the others, but no morphological differences were apparent. Such differences began to appear 24 h later at the prism or very early pluteus stage. In the mixed water these were more advanced with more prominent arm-buds than were any of those in the Clyde or Outside waters, although at this time there was little to choose in swimming vigour. On 1 May 1960, when 4 days old, the superiority of the larvae in the mixed water (all three dishes) was even more marked and they were again concentrating more to the surface than in the Clyde or Outside waters. The Clyde larvae were the worst with the shortest arms and the highest proportion of abnormals; the best of them resembled closely the 'Clyde-type' of the preceding experiments. In the Outside water the best larvae resembled the 'E 1-type'. The following day the larvae in the mixed water and in the Clyde water were nearly all dead. In the Outside water the larvae survived for another day or two, though all of them rapidly became very abnormal through loss of their arms.

The blastulae decanted into clean beakers (nos. 1 *a*–3 *a* in Table 4) at 22 h now claim attention. Two days later, on 30 April 1960, at the prism stage, most of those in the Outside water were resting on, or swimming slowly over, the bottom with very few swimming in mid-water. In both Clyde and mixed waters more larvae were swimming well up above the bottom than lying on it. This difference in swimming vigour persisted for another day or so. On 2 May 1960 the Clyde larvae, now 5 days old, were almost all down on the bottom but in the mixed water fair numbers were still swimming well above and continued to do so through 6 May 1960 and some even as late as 9 May 1960. The morphological differences were even more striking. In the Clyde water the larvae never grew arms and had irregularly formed, sometimes few and very small skeletal spicules. In the Outside water there was better though generally abnormal development of the skeleton and the best larvae were more pluteus-like, though no normal pluteus could be found. In the mixed water, although there was a big proportion of abnormal larvae, which were partially or completely armless, there were many larvae with long arms and strong though often abnormal skeletons, and a few quite normal plutei were present. These differences are brought out clearly in the photographs of mounted 9 days old larvae (Pl. III).

At the beginning of the experiment the room temperature was 17·5° C, it rose a degree and fell to 16° C in the first 24 h, thereafter varying slowly for several days between 15° and 18° C.

DISCUSSION

Our anticipation (p. 664) that the Clyde water would prove better than the water from E 1 was not fulfilled; on this occasion the reverse was true although neither water was particularly good, that is if we can assume that it was the waters and not the eggs which were to blame for the relatively few good plutei developing in any of the vessels, and for the early large mortalities in most dishes of Expts. 2 and 3. That it was not the eggs is perhaps indicated by the experience with dishes X and Y of Expt. 3, where almost all eggs kept continuously in mixed water developed normally for a few days while those in either water alone did not (p. 675). None the less Expt. 1 shows very clearly that each water so influenced the development that a type of larva peculiar to it, structurally different from that in the other water, was produced. This occurred irrespective of copper concentration up to and beyond the maximum ever likely to be found in a natural sea water, and exceeding the highest figure given by Atkins (1953) for the Plymouth district. Moreover, whilst it is true that in the higher copper concentrations the larvae were distinctly stunted compared with those in lower concentrations (so confirming this aspect of Bougis's work) the differences were not of the same character, or as great, as they have been in past experiments when two waters of different origin have contrasted strongly (as in Expts. 5 and 8, Wilson, 1951; Expt. 1, Wilson & Armstrong, 1952; Expt. 7, Wilson & Armstrong, 1958). Our 1960 experiments therefore do not confirm Bougis's thesis in so far as it attempts to explain much of our earlier work, for our results show that differences between waters exist despite varying copper concentrations. Also, within the natural limits of such concentrations the effect of copper on *Echinus* larvae is less than that which we have sometimes observed a natural water (after filtration) to have. But we agree with Bougis that copper in amounts found in natural sea waters affects developing larval structure to a lesser degree, and it is conceivable that on occasions it could be responsible for less fundamental differences between sea waters than those which we have from time to time encountered.

It is true that Bernhard (1957) working with *Arbacia lingula* showed a very marked difference between larvae of that species reared in water from the Gulf of Naples and in the same water after chelation. But as we have no direct comparison between the sensitivities to copper and other metallic ions of *Arbacia* larvae and those of *Echinus esculentus* it is not possible, for this and other reasons, to relate his observations directly to ours.

Both Bernhard and Bougis worked with species of sea-urchin different from ours and both conducted their experiments in manners not strictly

comparable with our methods. They made no comparisons between water from one locality and water from an oceanographically different water mass. They often did not continue their experiments for as many days. We ourselves have repeatedly shown that almost any addition to a water, or treatment of a water, has some effect, mild or otherwise, on *Echinus* larvae reared in it, though this may not become apparent until some days have elapsed. We are not surprised that addition of copper, or any other substance, to a water will alter its properties. So far the alteration has usually been in the direction of making a water worse. With us chelation has slightly worsened a water, though if our waters had been heavily contaminated with metallic ions it is possible that chelation would have affected some improvement, as Bernhard found with Naples water. (Unfortunately he does not give figures for copper or other metals in the natural water he collected for his work.) In fact chelation may only improve a water when there is heavy metallic contamination, for it will be remembered that in Expt. 1 (p. 668) the addition of a little extra copper to the E 1 water brought about a marked improvement, suggesting that the larvae benefit from the presence of a little copper in the water. Further experiments along these lines are desirable; the matter cannot on this one result be considered proved.

The salinities of our waters were not known until after the experiments had been completed. In the first two experiments the salinity difference was not great and was closely similar to that in Expt. 1 of 1952 (Wilson & Armstrong, 1952, p. 338), where the larvae did so much better in the less saline Clyde water than in the salter E 1 water. There have been marked differences between larvae when the salinities of the compared waters have been very close (e.g. Expts. 3, 8 A, B, Wilson, 1951). There have also been experiments with dissimilar salinities and no great differences between larvae (Expts. 1–3 and 5, Wilson & Armstrong, 1954). But never before have we used water with a salinity as low as that of the Clyde water in Expt. 3 of the present series. However, it was the E 1 water of normal salinity which in this experiment was so inimical to early larval development. We have no detailed observations of the effect of lowered salinity on the development of *E. esculentus* similar to those of Medes (1917) for *Arbacia punctulata*, but her results with the latter species would suggest that the lowered salinity of our Clyde water in Expt. 3 was not solely responsible for the armless, skeleton-less plutei it produced. We can find no evidence from all our past experiences that salinity within the range we have used can account for those major biological differences between sea waters which we have encountered.

A striking feature of the 1960 experiments was the superiority of mixed water compared with either component alone. This was shown once in Expt. 2 and twice in different ways in Expt. 3. That it was not shown in Expt. 1 could be due to that experiment having been stopped at 4 days, because except in dishes X and Y of Expt. 3, where eggs had been kept con-

tinuously in mixed water and not at any stage put into either component alone, the phenomenon did not appear until after 4 days. Never before in our experience has a mixed water proved superior to either alone. It has been usual for a mixed water to be better than the worst component, and sometimes nearly as good as the better component. Several possible explanations of these phenomena spring to mind, but in the present state of our knowledge there is little to be gained by speculation; we prefer at the present time to record our observations without attempting to account for them. To us it would seem that these observations, taken in conjunction with those of previous years, show that the whole subject is much more complex than previously thought, and that it is unlikely that one simple factor (such as copper concentration, bacteria, or the relative availability of one single compound needed for normal larval growth) will explain everything. It may well be that a number of factors are operative, sometimes one or more, sometimes others, varying from time to time and place to place. We must look not for one explanation but for many, and the task of tracking these down is likely to prove tedious and time-consuming. What cannot be denied is that waters are different, even if those differences show up only in laboratory experiments, although it would seem reasonable to conclude that they must also have their effects in the sea itself.

In another respect the 1960 experiments are of interest; they show once again that eggs can be affected by the water they are in between fertilization and first cleavage, when this water differs from that in which they subsequently develop. This was brought out in Expt. 3 with mixtures (see Table IV, Sets III–V), bearing in mind the manner of washing of the contained eggs, and also in the same Expt. with dishes X and Y. These experiences support similar observations in 1954 and 1955 (Wilson & Armstrong, 1958) using surface and deep waters collected at E 1.

SUMMARY

Sea water from the Plymouth district and sea water from the Firth of Clyde each produced, from the same batches of fertilized eggs of *Echinus esculentus*, a different morphological type of larva. Each type was clearly recognizable irrespective of the copper concentration up to a level higher than that ever likely to be found naturally in the localities from which sea waters contrasted in earlier experiments had been obtained. Suggestions by continental workers that these earlier results may have been due to varying copper concentration are thus shown to be inapplicable.

At higher levels of copper concentration larvae are stunted in their growth.

Chelation of the two waters slightly worsened them, confirming our earlier observations on the effect of this treatment.

In one of the waters to which a little extra copper had been added markedly improved larvae were obtained; this in conjunction with the effects of chelation

suggests that copper in small amount is beneficial. This point requires further investigation.

On three occasions much better larvae were obtained in mixtures of the two waters than in either water alone. Skeletal development was enhanced.

It was again found that subsequent development can be affected by the water the eggs are in between fertilization and first cleavage.

In conclusion it seems unlikely that one simple factor is responsible for biological differences between sea waters whenever and wherever they occur. It may be that a number of factors are operative, varying with time and place. The whole subject is probably more complex than previously thought.

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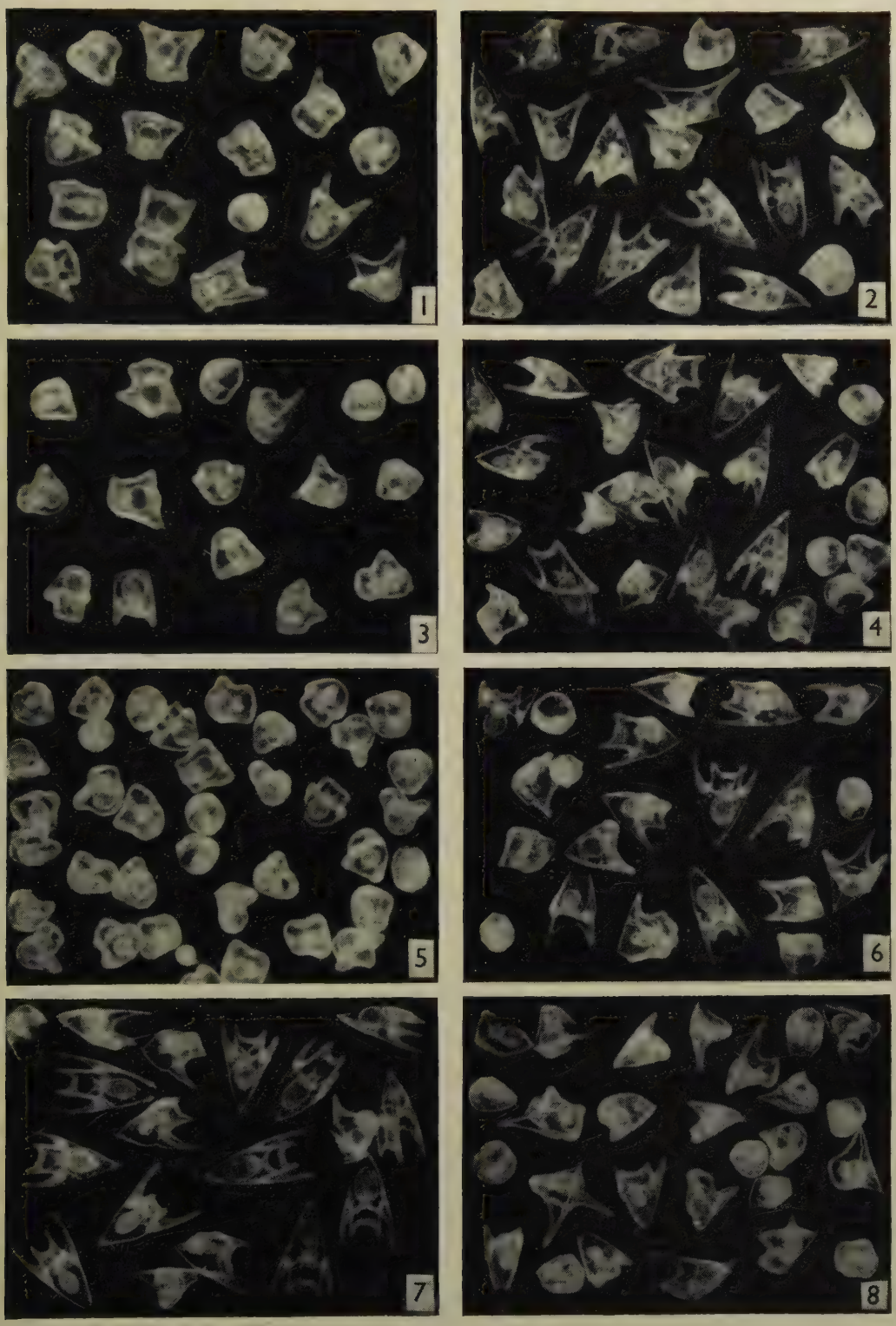

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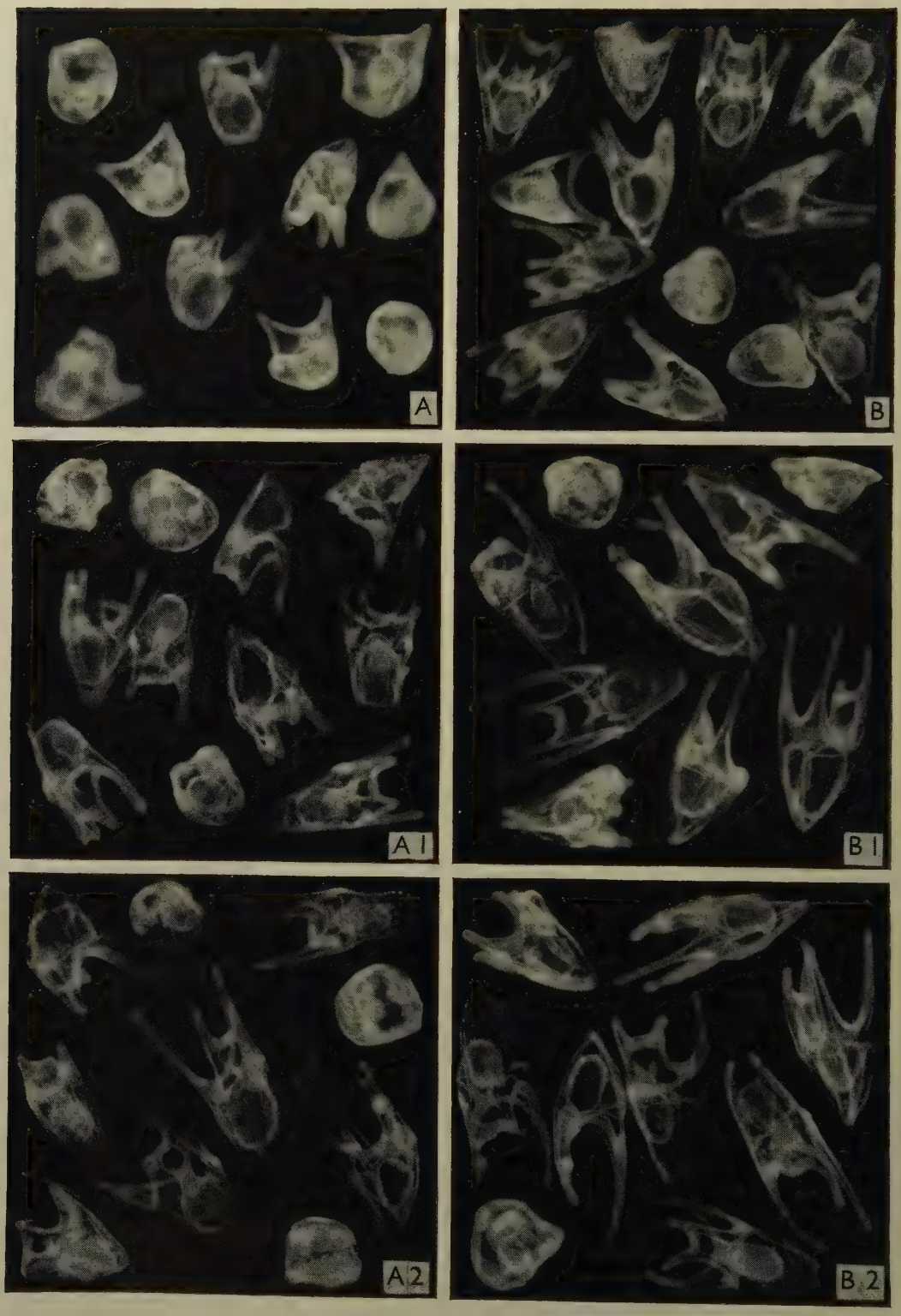
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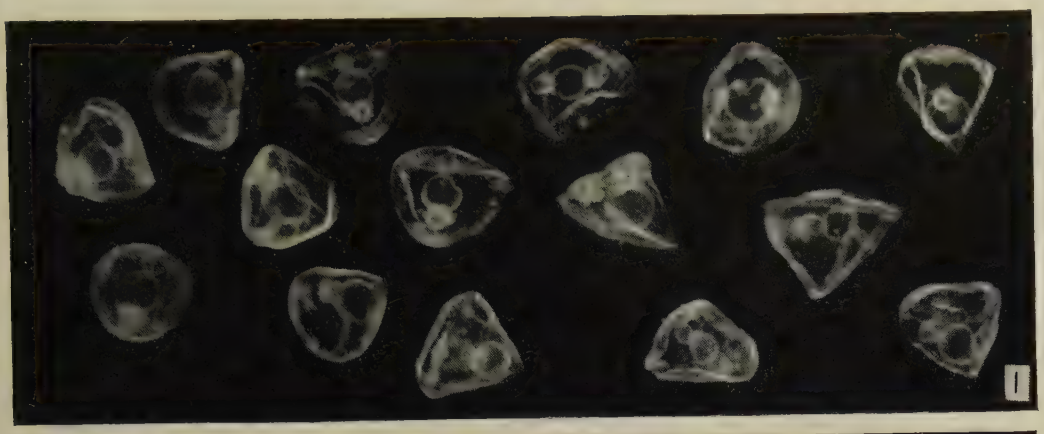
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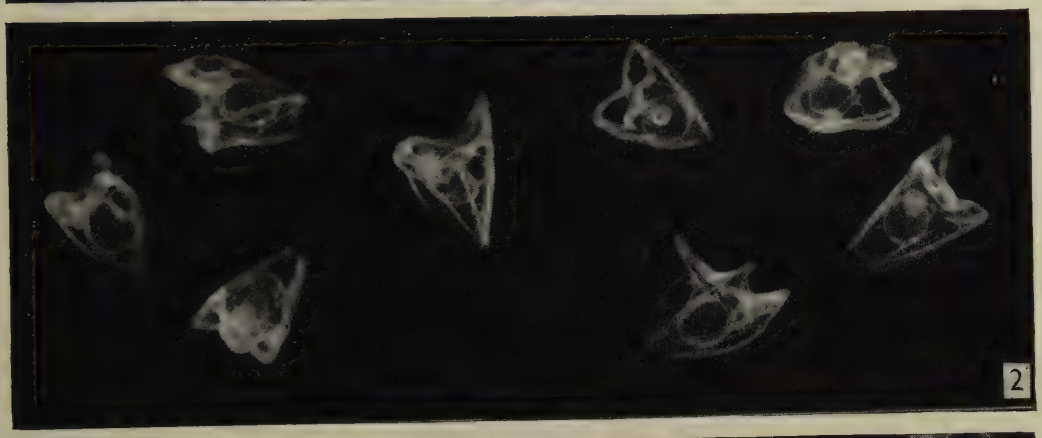
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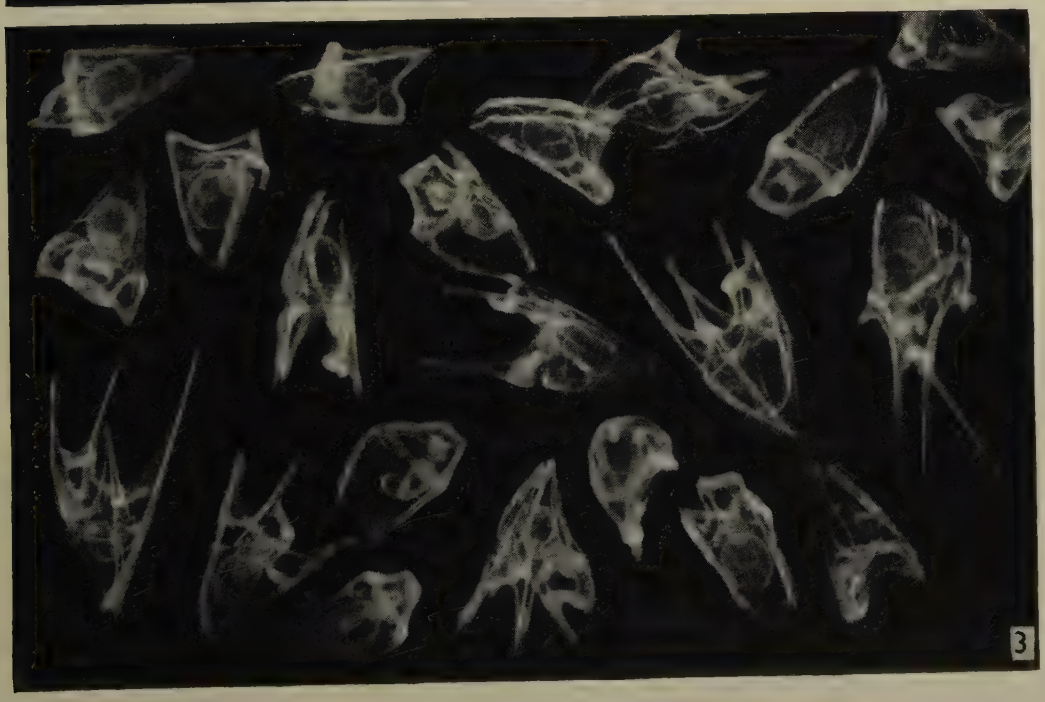
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EXPLANATION OF PLATES

(All photographs are of larvae fixed and preserved in alcohol and mounted unstained in Euparal. Photographed under dark-ground illumination. In order to save space several photographs have been combined by montage in each figure, but this has not involved any selection of good or bad larvae.)

Plate I

Larvae from Expt. 1. Fixed between 10 and 10.30 a.m., 26. iii. 60 (see p. 668). Magnification, ×35.

Fig. 1. Larvae from Set I. Clyde water; Cu, 1·5 μg/l.
Fig. 2. Larvae from Set II. E 1 water; Cu, 0·6 μg/l.
Fig. 3. Larvae from Set IV. Clyde water, chelated.
Fig. 4. Larvae from Set V. E 1 water, chelated.
Fig. 5. Larvae from Set VIII. Clyde water; Cu increased to 20 μg/l.
Fig. 6. Larvae from Set XI. E 1 water; Cu increased to 20 μg/l.
Fig. 7. Larvae from Set XIII. E 1 water; Cu increased to 1·5 μg/l.
Fig. 8. Larvae from Set XIV. Western water; Cu, 0·8 μg/l.

Plate II

Larvae from Expt. 2, beakers (see pp. 671–673 and Tables 2 and 3). Magnification, ×60.

Fig. A. Larvae from beaker A, Clyde water. Fixed 11 a.m. 4. iv. 60.
Fig. B. Larvae from beaker B, E 1 water. Fixed 11 a.m. 4. iv. 60.
Fig. A 1. Larvae from beaker A 1, Clyde water + Clyde water. Fixed 10.40 a.m. 7. iv. 60.
Fig. A 2. Larvae from beaker A 2, Clyde water + E 1 water. Fixed 10.37 a.m. 7. iv. 60.
Fig. B 1. Larvae from beaker B 1, E 1 water + E 1 water, Fixed 10.33 a.m. 7. iv. 60.
Fig. B 2. Larvae from beaker B 2, E 1 water + Clyde water. Fixed 10.30 a.m. 7. iv. 60.

Plate III

Larvae from Expt. 3, beakers. Fixed 3.20 p.m. 6. v. 60 (see p. 676 and Table 4). Magnification ×60.

Fig. 1. Larvae from beaker 1 *a*. Clyde water.
Fig. 2. Larvae from beaker 2 *a*. Outside water.
(N.B. Beaker 2 *a* contained fewer larvae than either of the other two. See p. 675).
Fig. 3. Larvae from beaker 3 *a*. Mixed water.

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DIFFERENCES REVEALED BY PAPER PARTITION CHROMATOGRAPHY BETWEEN THE GASTROPOD *NASSARIUS RETICULATUS* (L.) AND SPECIMENS BELIEVED TO BE *N. NITIDA* (JEFFREYS)

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(Plates I–III and Text-fig. 1)

During studies of the bottom fauna of the Essex oyster grounds a gastropod of the family Nassidae was found to be common. This mollusc was provisionally identified as *Nassarius reticulatus* (L.), but showed some characteristics which closely resembled those of *N. nitida* (Jeffreys) (Mistakidis, 1951, p. 19). In view of the uncertainty of the identification of the Essex specimens and the fact that Jeffreys (1867, p. 350), in giving the variety *nitida* specific rank, had expressed misgivings, Dr H. A. Cole and Mr M. N. Mistakidis initiated a more detailed study of the taxonomy of the Essex material. Their current unpublished work tends to show that, on morphological grounds, the Essex material should be ascribed to the species *N. nitida*. As part of this investigation the writer was asked to see whether paper chromatography would reveal any consistent biochemical differences between the Essex material and specimens from Plymouth, where the local species had been definitely identified as *N. reticulatus* (L.) (Marine Biological Association, 1957). The fact that *Nassarius* found off North Wales resemble the Plymouth specimens morphologically, and are ascribed to the species *reticulatus*, was also utilized. The present report sets out the result of this biochemical study.

The usefulness of the technique of paper partition chromatography in the field of systematics was reviewed by Farris (1957), while a very useful review, comprehensive as regards zoological studies, has been made by Wright (1959) in the introductory section of his paper dealing with the biochemical specificity of snail mucus. A recent paper by Alston & Turner (1959) gives further references, while work done on bacteria by several investigators has been outlined by Mattick, Cheeseman, Berridge & Bottazzi (1956).

Judging from the results of the workers who have used this method, it would seem that, with certain reservations, the biochemical complex of each species does indeed produce its own characteristic pattern when chromatographically separated. Very good evidence for the validity of the method has been produced by Alston & Turner (1959), who found that they could invariably distinguish *Baptisia laevicaulis* from *B. viridis* by chromatography;

each gave a constant pattern, while hybrids gave many intermediate patterns. Further supporting evidence comes from work carried out at the National Institute for Research in Dairying and published in a series of papers (Mattick *et al.*, 1956; Berridge, Cheeseman, Mattick & Bottazzi, 1957; Cheeseman, Berridge, Mattick & Bottazzi, 1957; Gregory & Mabbitt, 1957*a*, *b*; Mattick, Berridge, Cheeseman & Mabbitt, 1957). These investigators found that, while different bacterial species gave different free amino-acid and peptide patterns, different strains of the same species gave substantially the same pattern; there were, however, some variations, less than those between species, due to age of culture or to composition of medium. Micks (1954) found chromatography useful in separating mosquito species, and Buzzati-Traverso & Rechnitzer (1953) found the fluorescent and ninhydrin-positive patterns produced by fish muscle to be remarkably constant within a species, regardless of size or age of the individuals, while different species produced constantly distinct patterns, the differences being greater the more distantly related the species under comparison. Wright (1959) found constant patterns to be produced by mucus of several species of *Lymnaea*, but in some cases the extent of similarities of pattern did not appear to be correlated with degree of relationship between the species. Such a correlation was, however, indicated when foot tissue of Australian land snails was used (Kirk, Main & Beyer, 1954). Further examples are to be found in the reviews to which reference has already been made.

From a study of all the available results, one is led to the general conclusion that each species produces its own characteristic chromatographic pattern; however, as has already been mentioned with respect to bacteria, it has also been found that individuals of the same species do not always produce exactly the same pattern. It is therefore essential that careful control in experimental methods and great caution in interpretation be exercised—a point made by both Farris (1957) and Wright (1959), in whose reviews further examples of such variation can be found. Different diets, and also starvation, affect the pattern in *Sardinops caerulea* (Farris, 1958), and the present paper describes an effect due to starvation. In *Theba pisana* three different diets had no effect on the pattern (Kirk *et al.*, 1954). Again, it is obvious that, when comparing a number of organisms by this method, the corresponding tissue must be taken from each, or, alternatively, as in the investigation using whole mosquitos, exactly the same combination of organs. Different tissues from the same organism will give different patterns. Such a study on *Phaseolus vulgaris* seedlings was described by Greenshields (1958). Wright (1959) found a seasonal variation in laboratory-bred *Lymnaea palustris*. Metamorphosis also causes changes, but it would be surprising if such a biochemical upheaval did not do so.

On the other hand, the possibility that two different species might produce the same pattern cannot be completely excluded, though on theoretical

grounds a unique pattern for each species would be expected, provided that a wide enough range of compounds had been investigated.

MATERIAL

Specimens of the Essex form of *Nassarius* were collected from the Rivers Crouch and Roach, and *N. reticulatus* samples were collected off Plymouth. Those animals which were not used immediately on arrival were kept in running sea water in the laboratory. The glass tanks containing the two different forms were kept under identical conditions; where feeding was involved, individuals of the two batches were placed in the same tank after being marked on the shell with different coloured paints in the way described by Hancock & Urquhart (1959). Mussels and crab meat were used for food.

For the supplementary investigation involving a small number of North Wales specimens, the animals were collected at Rhoscolyn, Anglesey.

Littorina were obtained from the Thames estuary and the Crouch.

The tissue used was that of the posterior end of the foot. Differences in the chemical composition of this organ are likely to correspond with true differences in body composition, rather than merely to reflect recently absorbed substances. The piece of tissue was washed in distilled water immediately after detachment, and prepared at once for paper chromatography.

CHROMATOGRAPHIC METHODS

Whatman No. 3MM chromatography paper was used. Sheets 42 cm wide were divided into eleven columns for the main investigation; some of the preliminary tests were made on narrower sheets. Length from start-line to base was also 42 cm. The descending technique was employed.

In the method finally adopted, about 5 mg foot tissue was used from each individual. Each column on the paper represented a different individual, and the tissue sample was applied to the start-line as a streak about 1·5 cm long, centrally in the 3·8 cm wide column. The paper was placed on clean glass and the tissue squashed in well by means of the flat base of a small specimen tube. The tissue streaks were thoroughly dried in a stream of hot air. In preparing papers for examination by the ultra-violet method, 15–40 mg of tissue were crushed and extracted in a very small volume of hot 70% ethanol. The extract was applied to the start-line and dried.

The solvent used was that found most suitable for a similar investigation on land snails by Kirk *et al.* (1954); i.e. *n*-butanol:acetic acid:water (100:22:50 v/v). No other solvent mixture was tried.

Preliminary experiments were made to ascertain the time necessary for maximum separation without loss of ninhydrin-positive or ultra-violet fluorescing material from the bottom of the paper. A satisfactory time was found to be 30–40 h according to room temperature, there being no temperature control.

Different methods were tried for the examination of the dried papers. The most useful method was found to be the application in the form of a spray of

0·1 % ninhydrin in water-saturated butanol; the paper was then redried in a warm air stream and further heated at 100° C for 10 min. Examination in ultra-violet light (3660 Å), which was tried as an alternative method, revealed little until refinements were made in the application of the tissue material to the paper, as described above. Then, on recently heated papers, moderately well defined spots could be observed, following a pattern very suggestive of the ninhydrin-positive pattern. The fluorescent spots were outlined in pencil, and the papers then treated by the preceding method for revelation of ninhydrin-positive substances. Very well formed spots appeared, exactly coinciding with the marked fluorescent areas, which would therefore seem to be due solely to amino-acids. Although the method of applying tissue substances as an alcoholic extract resulted in much sharper spots, this was not used for the main investigation. The greater time involved was not felt to be justified, as the revealed ninhydrin-positive pattern was the same in both cases. The production of denser, better defined spots was undoubtedly due to the fact that a larger quantity of the active substances was applied to a smaller area of paper in the form of an extract than as whole tissue, the amino-acids therefore running in higher concentrations to smaller areas.

PRELIMINARY EXPERIMENTS

Preliminary experiments were planned first to throw light upon the more serious of the two points of doubt outlined above concerning the reliability of the technique, in so far as it affected this investigation. It was essential to know the extent of individual variation within an apparently homogeneous batch, so before attempting to compare Essex with Plymouth specimens of *Nassarius*, the variability within the Essex form was investigated. The effects on the ninhydrin-positive pattern of (*a*) size of individual, (*b*) exact location of collection, and (*c*) starvation *versus* feeding, were ascertained.

The size of the individual had no effect on the chromatographic pattern, the only exception to this being that with very small individuals one or two of the pattern spots were sometimes difficult or impossible to see. The amount of tissue which could be taken from these specimens without encroaching on other organs was considerably smaller than the standard amount, and the chromatographic result was very probably due to the use of insufficient material and not to any real difference. As confirmation of this, a similarly small amount of tissue from a large individual was chromatographed, producing the same effect, which was therefore ignored; but very small specimens were not used in the comparative work.

Individuals differing from one another only in that they were collected from four different locations in the Essex rivers exhibited no differences in pattern.

Duration of starvation, however, had an appreciable effect on the ninhydrin-positive substances of the foot tissue. Preliminary studies established that a consistent pattern was produced by different individuals from the same area when recently fed; and that a different but related pattern, equally reproducible, resulted from individuals which had been starved. Short periods without food resulted in intermediate patterns, as the changes occurring during starvation were due to the gradual disappearance or reduction of certain of the spots. The changes, which are referred to in greater detail in the next section, were reversible, even after prolonged starvation.

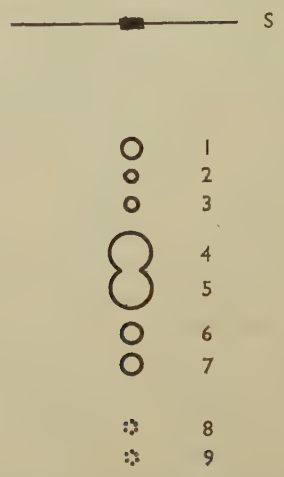
The theory that two definitely different species always produce different chromatographic patterns—identical patterns always indicating individuals of the same species—invited exploration, but could obviously provide an unlimited subject of study. As, in fact, the two forms of *Nassarius* did not produce identical patterns, this, the second point of doubt referred to above, was not very relevant to this investigation. However, tests were made to assess the degree of difference which might be encountered between species of two genera which were neither very closely nor too distantly related, and the results obtained may be of sufficient interest for inclusion here. *Littorina littorea* (L.) was selected as a readily obtainable marine gastropod of the same order of size as *Nassarius*. This species produced a consistent pattern which did not vary with the size of the animal, the place of collection, or with starvation, and which, though it also had much in common, differed distinctly from the *Nassarius* pattern. The *Littorina* pattern is described on p. 691.

CHROMATOGRAPHIC COMPARISON BETWEEN ESSEX AND PLYMOUTH SPECIMENS

Because duration of starvation had been found to have an appreciable effect on the ninhydrin-positive substances of the foot tissue, the main experiments involving comparisons between the Plymouth and Essex specimens were planned to follow changes over a period of time after removal from their natural habitats. Specimens starved for a known period were used and also some which had subsequently been fed. In this way it was hoped that the immediate effects due to differences in the two natural environments would be eliminated, and that any differences revealed would therefore be of genetic origin.

Also with the aim of equalizing as many factors as possible, the collections of a sample at Plymouth and one from the River Crouch were made on the same day. Both samples were similarly boxed, and unpacked at the same time when the Plymouth specimens arrived at Burnham-on-Crouch 2 days later. These exactly parallel specimens were used for many of the chromatograms, including those shown in Pl. I, fig. 2 and Pl. II, fig. 2. When applying the

prepared tissue or extract to the paper, material representing Plymouth individuals was always placed in columns alternating with those occupied by material from Essex specimens.



Text-fig. 1. Diagrammatic representation of *Nassarius* ninhydrin-positive pattern, showing the numbering of spots (1 to 9) as used in the text. S, start-line and insoluble part of applied tissue.

As the solvent front reached the edge of the paper after the elapse of about one-third of the optimum development time, no R_F data are available. To facilitate the description of results, the ninhydrin-revealed spots have been numbered, beginning with the one running most slowly. Reference should be made to Text-fig. 1. Spots 6 and 7 proved to be useful 'markers', being quite constant in all the *Nassarius* and *Littorina* specimens. As the solvent did not flow at the same rate at all points across the width of the paper, this was a valuable feature.

No attempt was made to identify the substances, presumed to be amino-acids, responsible for the ninhydrin-positive pattern. Spot 5 was bluish purple on fresh chromatograms, the remaining spots being shades of reddish purple. Spot 1 remained as a faint brown spot months after the others had completely faded. It appeared brownish purple on fresh chromatograms when held up to the light. It was also found to react differently from the others in that its original purple colour turned blue when the chromatograms were stored with papers which had been sprayed with *p*-anisidine hydrochloride. Unsprayed chromatograms viewed in ultra-violet light showed Spot 4 as a slightly more bluish fluorescence than the others.

The results are illustrated in Pls. I–III. These chromatograms are necessarily only a selection, as many others were run, but the results were strikingly reproducible. In all, the number of individuals used for this work, including

preliminary experiments, amounted to: Essex specimens of *Nassarius*, 188; Plymouth specimens, 128; North Wales specimens, 11; *Littorina*, 20. The following points become clear from the study of these and other ninhydrin-sprayed chromatograms.

Spot 1

Essex Consistently present, not diminishing with starvation.

Plymouth Never as well marked as in Essex specimens. Disappeared gradually on starvation. Reappeared after feeding.

Spot 2

Behaviour not consistent enough for use in separation. Often the first spot to disappear in starving Essex specimens, though it sometimes persisted for a considerable time. Disappeared quickly in starving Plymouth specimens, and was never so obvious in a Plymouth specimen as in a parallel Essex one.

Spot 3

Essex Disappeared very slowly during starvation, reappearing after feeding.

Plymouth Behaved like Spot 1.

Spots 4 *and* 5

Essex 5 larger than 4, 4 becoming even less evident during starvation, while 5 remained constant.

Plymouth 4 and 5 sometimes equal or nearly equal; but more usually 4 was distinctly larger than 5, this invariably indicating a Plymouth individual. Effect of starvation slight, being seen as a tendency for 4 to be distinctly larger than 5 in a higher proportion of fresh than starved specimens.

The proportions of the double spot which should be allocated to 4 and 5 respectively were much more readily seen in the original chromatograms, where the different shades of reddish and bluish purple could be easily distinguished, than in photographs. There was some evidence that further development might have divided Spot 5, and possibly also Spot 4, into more than one component. In particular, in well developed chromatograms, a small faster-running area seemed to be separating from the large main part of Essex Spot 5. When alcoholic extracts were chromatographed, ninhydrin-revealed Spot 4 was relatively more evident, Spots 4 and 5 appearing nearly equal in Essex specimens and Spot 4 (appearing to consist of two components) always distinctly larger than 5 in Plymouth specimens.

Spots 6 *and* 7

Essex and Plymouth. Consistently present.

Spots 8 *and* 9

These two spots were so faint that they could not be used critically, and were often allowed to run off the paper. They had begun to disappear from some individuals after 1 week without food.

CHROMATOGRAPHIC PATTERN GIVEN BY NORTH WALES SPECIMENS

As the chromatographically different Plymouth and Essex *Nassarius* differed both morphologically and in their area of origin, a desirable supplementary investigation was a chromatographic comparison between batches of the *N. reticulatus* form from widely separated areas representing somewhat different habitats. To this end a few North Wales specimens were run parallel to Essex specimens in the same way that the Plymouth ones had been. Tissue squashes of the North Wales specimens were made by the standard technique soon after the animals were taken from the sea, and the papers were dried thoroughly and stored until tissue from similarly fresh Essex specimens had been applied to alternate columns of the same papers. Experience with Essex animals had shown that, when kept thoroughly dry, storage at this stage had no effect on the chromatographic pattern, and this fact was also mentioned by Wright (1959), and by Enge & McKee (1959). The pattern produced by the North Wales specimens could not be distinguished from that of Plymouth specimens which had been used as soon as received, but was readily distinguishable from the parallel Essex pattern (Pl. I, fig. 1). North Wales specimens were not chromatographed after periods of starvation.

EXPLANATION OF PLATES I–III

Ninhydrin-sprayed chromatograms, showing patterns produced by the foot tissue of specimens of *Nassarius* and *Littorina littorea*. Alternate columns of all chromatograms, except that shown in Pl. III, fig. 2, represent *Nassarius* individuals from Essex and Plymouth (or North Wales), respectively. The letters 'E', 'P' and 'W' have been inserted beneath the columns to indicate the place of origin of each specimen.

Plate I

Fig. 1. Samples, taken shortly after collection, from Essex (E) and North Wales (W) specimens. The North Wales pattern shown here was identical with that of Plymouth specimens used as soon as received.

Fig. 2. Samples taken 1 week after collection, starved. Chromatograms of starved specimens sampled 2 weeks after collection were very similar in appearance to this.

Plate II

Fig. 1. Plymouth (P) specimens starved 9½ weeks; Essex (E) specimens starved 8½ weeks. (Duration of starvation probably sufficient to allow the difference of one week to be ignored.)

Fig. 2. Samples taken after 3 weeks' starvation and subsequent feeding.

Plate III

Fig. 1. Plymouth specimens starved 10½ weeks, Essex specimens starved 9½ weeks, both batches subsequently fed.

Fig. 2. *Littorina littorea* (left-hand column) and Essex *Nassarius* (remaining columns). In this preliminary test the paper was somewhat overloaded, resulting in rather pronounced streaks between the spots.

Fig. 1

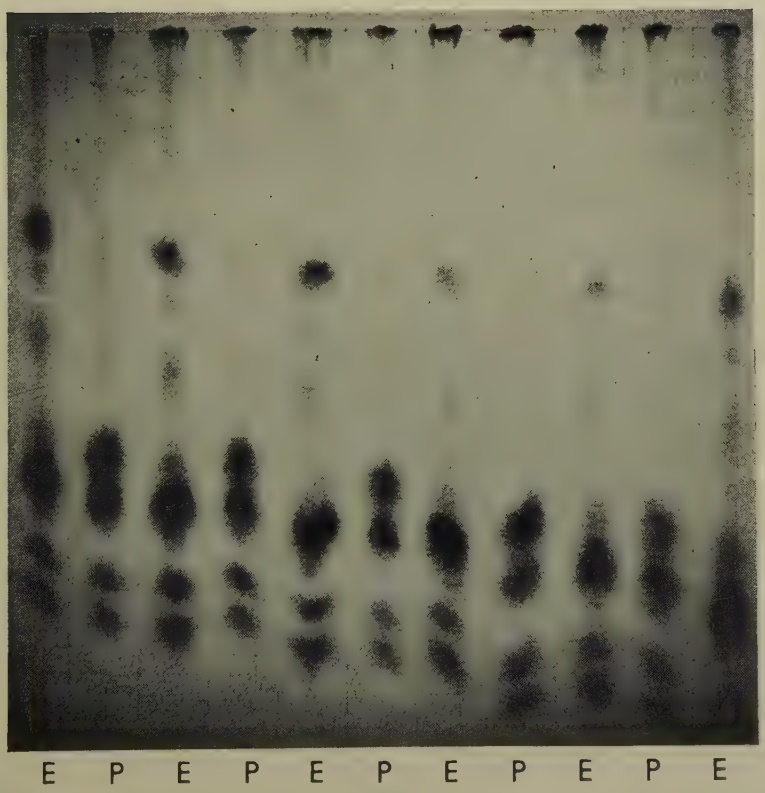


Fig. 2

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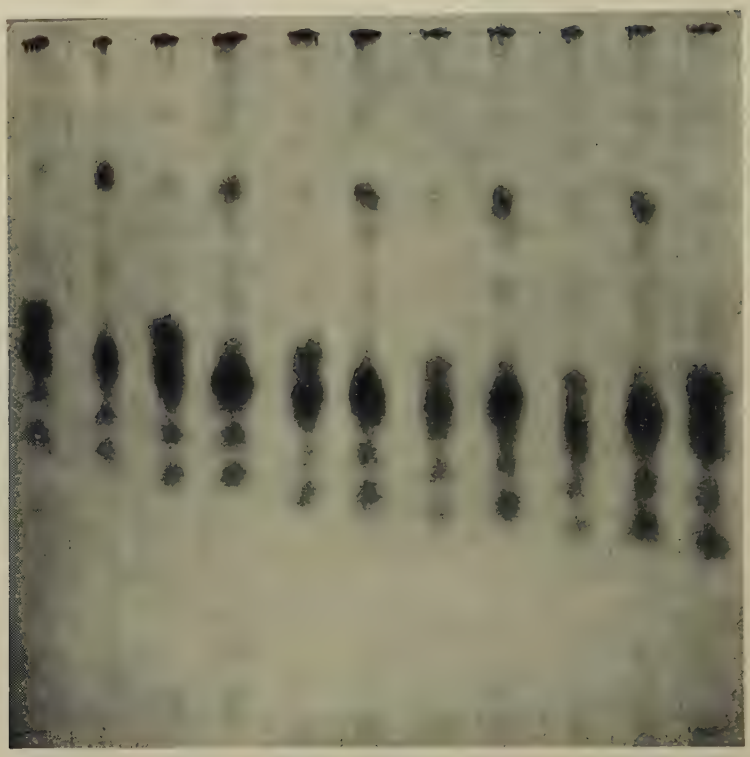

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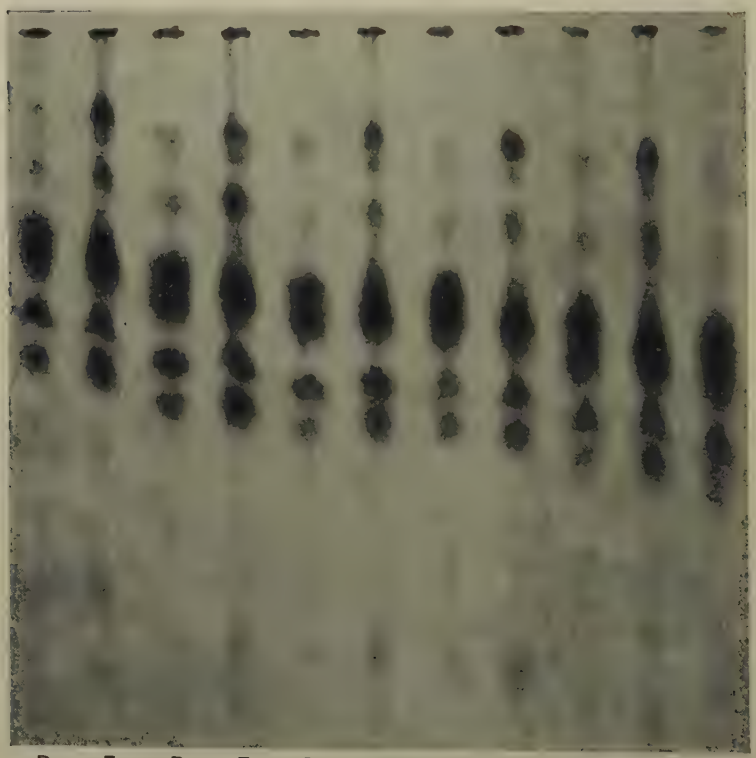
Fig. 1



P E P E P E P E P E P

Fig. 2

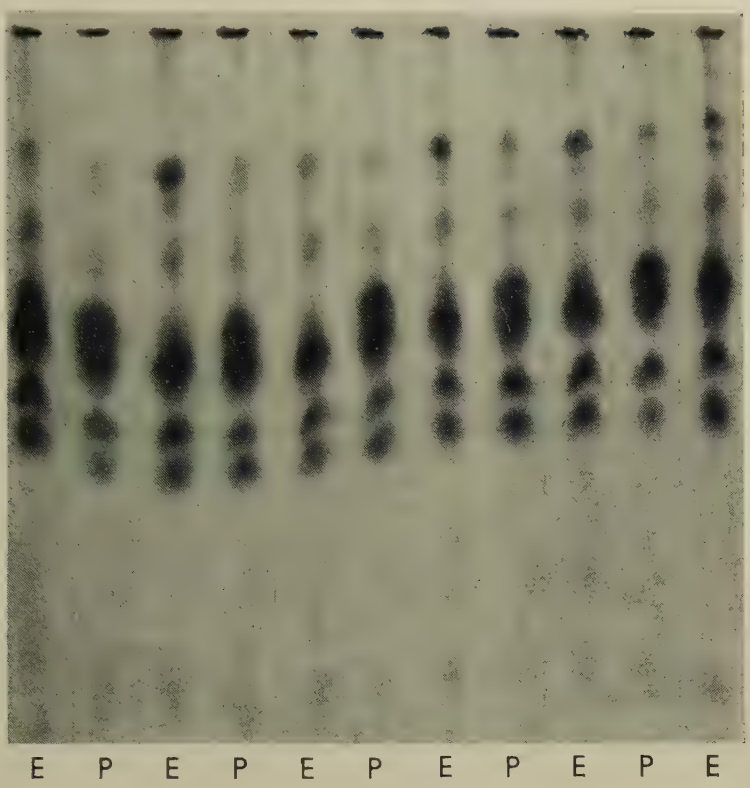


Fig. 1

Fig. 2

CHROMATOGRAPHIC PATTERN GIVEN BY *LITTORINA LITTOREA* (L.)

The degree of difference between the two forms of *Nassarius* may be compared with the one example of inter-generic variation which was assessed. *Littorina littorea* never exhibited a spot at positions 1 or 2, but sometimes a trace was just visible near position 3. A double—possibly triple—spot corresponded to 4 and 5. Spots 6 and 7 were always present, while one or two more rapidly running spots, not corresponding to 8 or 9, were just visible in some instances. The *L. littorea* pattern is illustrated in Pl. III, fig. 2, left-hand column.

DISCUSSION

The results show that there were undoubtedly reproducible differences between the Essex and Plymouth specimens of *Nassarius* used for this investigation, and in view of the precautions taken to eliminate environmental factors it is concluded that these are evidence of genetic differences between the specimens of *Nassarius* from the two areas. The supplementary investigation using North Wales specimens adds weight to the conclusion that the Essex form is really different from the *reticulatus* form.

Although the Essex specimens are thus fairly certainly genetically different from the Plymouth and North Wales specimens, it is not at present possible, from the chromatographic evidence alone, to ascribe to them any particular degree of relationship. It would, for instance, be unsafe to deduce their degree of relationship from a comparison of the observed differences between the two forms on the one hand with the chromatographic differences between members of the two unquestionably different genera, *Nassarius* and *Littorina*, on the other, for without a comparison of the chromatographic patterns produced by several classes of chemical compounds of many closely related organisms it is impossible to give any estimate of degree and range of chromatographic difference associated with accepted variety, species or genus. Such a comparison is far beyond the scope of this investigation, but might well be rewarding. Indications of this are already to be found in the literature, and anomalies may disappear as further classes of compounds are included in the studies. Though speculation on the eventual deduction of a yardstick for the measurement of comparative differences and the degree of relationship which they represent is premature, it is felt that the results of this investigation add further weight to the claims, explicit or implied, of previous writers (see above) that paper partition chromatography is becoming an extremely useful tool in the sphere of systematics.

I wish to express sincere thanks to Dr F. S. Russell of the Plymouth Laboratory, Mr A. C. Simpson and others of this laboratory, and Mr P. R. Walne of the Fisheries Experiment Station, Conway, for arranging a supply of specimens from the Plymouth, Essex and North Wales areas respectively;

also to Mr P. J. Warren for taking the photographs; and to Dr L. Fowden of University College, London, for allowing me to use his ultra-violet lamp and for advice on the preparation of chromatograms for the ultra-violet method of examination. Special thanks are also due to Mr Simpson for most helpful criticism at the manuscript stage.

SUMMARY

The ninhydrin-positive patterns produced by chromatography of foot tissue of *Nassarius reticulatus* from Plymouth and North Wales and of specimens of *Nassarius* from Essex were established. The similarities and differences between the two are described and the consistent differences found are considered to be indicative of genetic differences between the specimens from Essex and those from the other two areas. The only factor found to affect the otherwise remarkably reproducible pattern given by individuals from the same area was the degree of starvation; the effect was reversible. The pattern given by another marine gastropod, *Littorina littorea* (L.), is also described. Reference is made to the potentialities of the technique.

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PHOTOMECHANICAL CHANGES IN THE EYES OF FISHES

I. RETINOMOTOR CHANGES IN *SOLEA SOLEA*

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(Plates I and II)

In many vertebrates (except Mammalia) cellular movements take place in the pigment epithelium and receptor layer of the retina in response to changes of illumination. These movements, known as retinomotor changes, are especially well marked in teleost fishes. In fishes exposed to light, the retinal pigment moves outwards towards the external limiting membrane, the cone myoids contract causing the cones to shorten towards the light, and the rod myoids elongate causing the rods to extend away from the light. Reverse changes take place in dim light and darkness. These responses are characteristic of fishes living in illuminated waters. Cited exceptions are eels, in which the cones are static, and flatfish, in which only the pigment moves whereas rods and cones are stationary (Garten, 1907; Parker, 1932; Bayliss, Lythgoe & Tansley, 1936; Walls, 1942). The present investigation deals with the pattern of retinomotor changes in the common sole *Solea solea* (L.).

MATERIALS AND METHODS

Soles were held in a tank of circulating sea water at 15° C. The room was light-proof and the tank could be illuminated by two 100 W bulbs directly overhead (distance 0·7 m). Soles were placed in the tank in the evening and lights were turned on next day, if desired. At the conclusion of the experiment fish were decapitated, corneas were pricked, and the heads were dropped into Bouin's solution. Eyes were sectioned in celloidin or wax and were stained with haematoxylin and eosin plus Biebrich scarlet. Sections were cut dorso-ventrally, through the centre of the eye. Bleaching was carried out with potassium permanganate followed by oxalic acid.

At least three fish were used for each experiment. Experiments were as follows:

(1) Dark-adapted. In dark 16.30–09.30 h. 17 h in darkness.
(2) Dark-adapted. In dark 17.00–12.00 h. 19 h in darkness.
(3) Dark-adapted. In dark 17.00 h (first day) to 24.00 h (second day). 31 h in darkness.

(4) Dark-adapted. In dark 17.00–06.00 h. Illuminated $8\frac{1}{2}$ h, 06.00–1430 h. Then treated:

(*a*) darkness $\frac{1}{2}$ h, 14.30–15.00 h.
(*b*) darkness 1 h, 14.30–15.30 h.
(*c*) darkness, 2 h, 14.30–16.30 h.

(5) Light-adapted. In darkness 16.30–09.30 h. Then treated:

(*a*) illuminated $\frac{1}{2}$ h, 09.30–10.00 h.
(*b*) illuminated 1 h, 09.30–10.30 h.
(*c*) illuminated 2 h, 09.30–11.30 h.

When a pigment index is used it has the following significance: 1, pigment basal, fully retracted; 2, $\frac{1}{4}$ expanded; 3, $\frac{1}{2}$ expanded; 4, $\frac{3}{4}$ expanded; 5, fully expanded (or extended).

OBSERVATIONS

Retinal pigment

Dark-adapted eyes. The retinal pigment was partially or wholly retracted in fish which had been in darkness (Pl. I, fig. 1).

(1, 2, 3) Darkness 17, 19, 31 h. The pigment was wholly or largely withdrawn into basal regions of the cells. Dorsal and central retina, index 1. In the ventral retina it was sometimes partially expanded (indices, 1, 2, 3). There was no difference between animals killed at 12.00 h and 24.00 h.

(4*a*) Darkness $\frac{1}{2}$ h. Pigment was partially or wholly retracted (partially extended in two specimens; fully retracted in four specimens).

(4*b*) Darkness 1 h. Pigment was retracted (indices 1 or 2).

(4*c*) Darkness 2 h. Pigment was retracted (index 1).

Light-adapted eyes. The pigment expanded in the light (Pl. II, fig. 1).

(5*a*) Light $\frac{1}{2}$ h. Dorsally and centrally the pigment was partially extended but thin (indices 2, 3, 4). Ventrally it was extended (indices, 4, 5) and thin to dense.

(5*b*, *c*) Light 1, 2 h. The pigment was extended to a lesser extent dorsally and centrally than ventrally. Mean dorsal index 3·1; mean central index 3·8. The pigment tended to be thin, especially dorsally. Ventrally the pigment was extended (indices 4–5) and usually dense.

Cones

Dark-adapted eyes. Cones tended to elongate in darkness (Pl. I, figs. 1, 2).

(1, 2, 3) Darkness 17, 19, 31 h. In the dorsal and central fundus cones were usually fully extended (7 fish), or a fraction were extended and a fraction contracted (2 fish). In the ventral field cones were fully extended in 4 fish; part extended, part retracted (1 fish); retracted in 4 fish.

(4*a*, *b*, *c*) Darkness $\frac{1}{2}$, 1, 2 h. Cones were extended in 7 fish; part extended, part retracted in 1 fish (2 h); retracted in 1 fish (1 h).

Light-adapted eyes. The cones were usually retracted in the light (Pl. II, figs. 1, 2).

(5*a*) Light $\frac{1}{2}$ h. Cones were partially or fully retracted. In the central and ventral retinae of two specimens some cones were retracted, others extended.

(5*b*) Light 1 h. Cones were mostly retracted. In the ventral retina of one specimen some cones were extended, others retracted.

(5*c*) Light 2 h. Cones were retracted.

Rods

Rods appeared to be static, but the preparations were not really suitable for following their movements, if any.

CONCLUSIONS

In the common sole the retinal pigment moves towards the external limiting membrane in the light, and towards the chorioid in the dark. Usually cones shorten in the light and elongate in darkness, but there are many exceptions, movement in the expected direction often being partial or wanting. In the light-adapted eye the pigment extends along the processes of the pigment cells and screens the outer segments of the rods (Pl. II, fig. 1). The pigment extends to the ellipsoids of the retracted cones, sometimes reaches the external limiting membrane, and comes to lie between the outer segments of the cones which are screened, to some extent from one another. Movements of pigment and cones occur in $\frac{1}{2}$–1 h.

No consistent difference was noted in pigment and cones of fish killed at 12.00 h and fish killed at 24.00 h, both groups being dark-adapted. The reverse experiment, of keeping fish in light by day and night, was not tried.

In dark-adapted eyes the pigment is sometimes slightly expanded in the ventral retina when it is fully retracted in the dorsal retina. Conversely, in light-adapted eyes, the pigment is sometimes denser and extended to a greater degree in the ventral retina compared with the dorsal retina. Also movement of cones tends to be retarded to a greater degree in the ventral retina. These differences may be connected with polarity in the eye (cf. Vilter & Thibault, 1948).

SUMMARY

In the common sole *Solea solea* retinomotor changes take place. The retinal pigment usually expands in the light and cones shorten; reverse changes take place in darkness.

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EXPLANATION OF PLATES I AND II

Sections through central retinae of *Solea solea* (magnification × 472. *e*, external limiting membrane; *c*, cones; *n.*, outer nuclear layer; *p*, pigment; *r*, rods).

PLATE I

Dark-adapted fish (2 h, 14.30–16.30 h)

Fig. 1. Pigment retracted, cones extended.

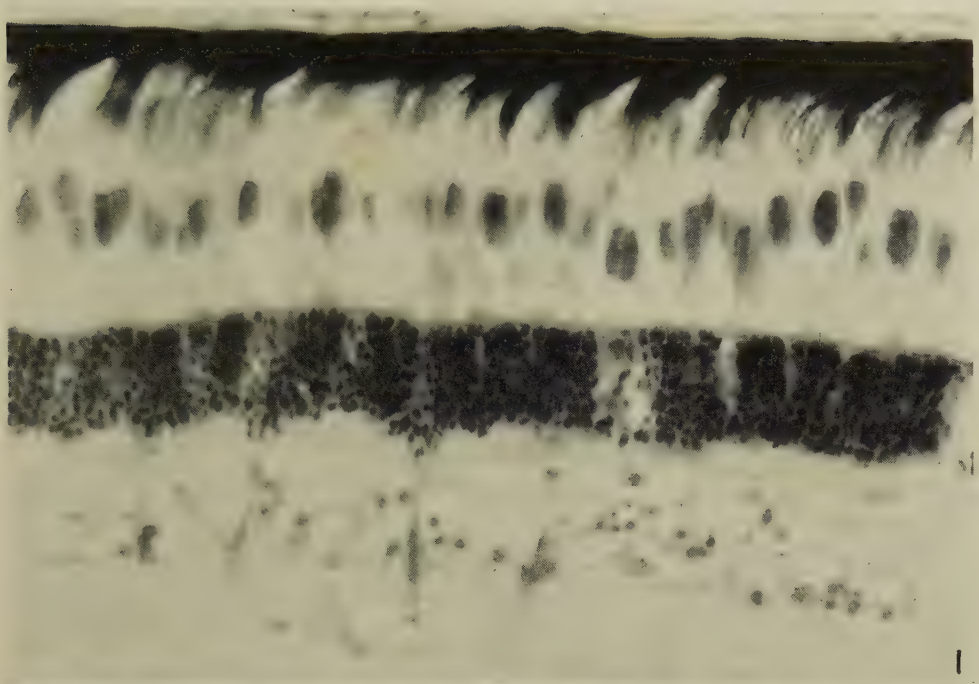
Fig. 2. Same, bleached, cones extended.

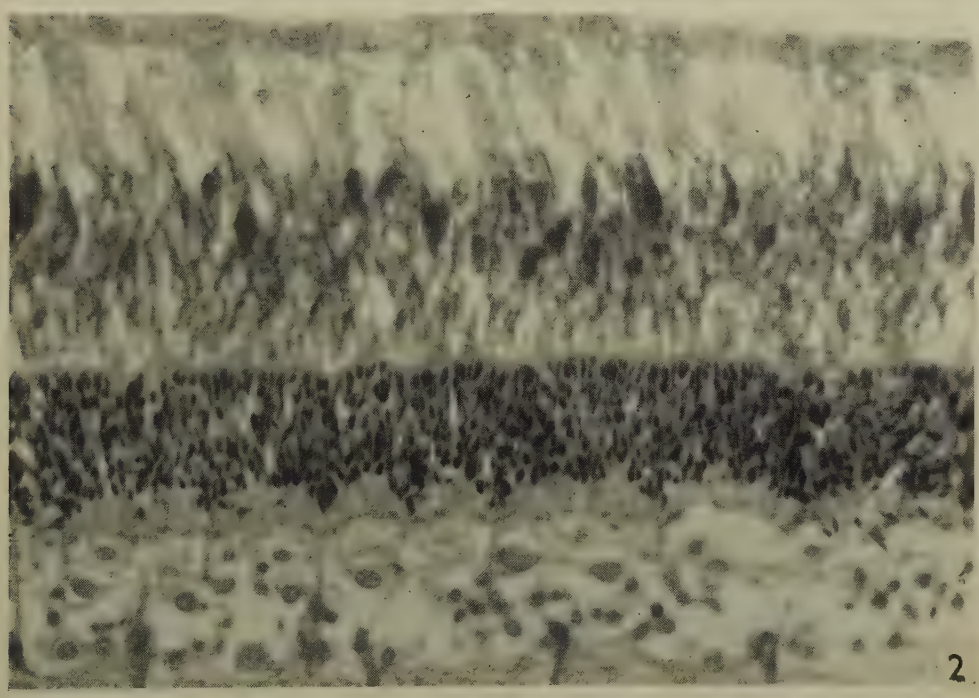
PLATE II

Light-adapted fish (2 h, 09.30–11.30 h)

Fig. 1. Pigment extended, cones retracted.

Fig. 2. Same, bleached, cones retracted.





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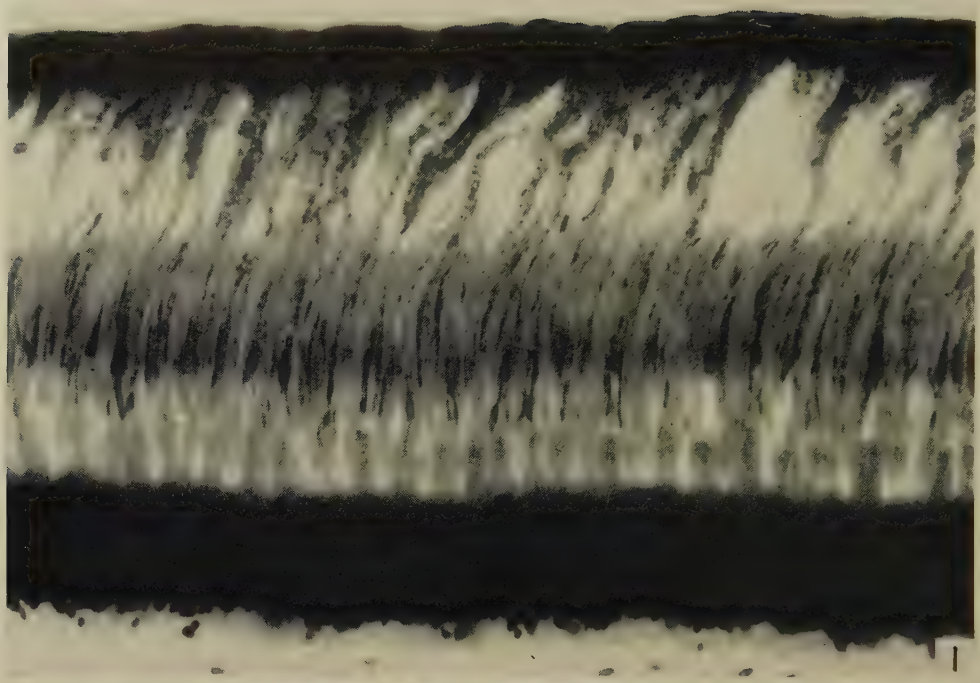
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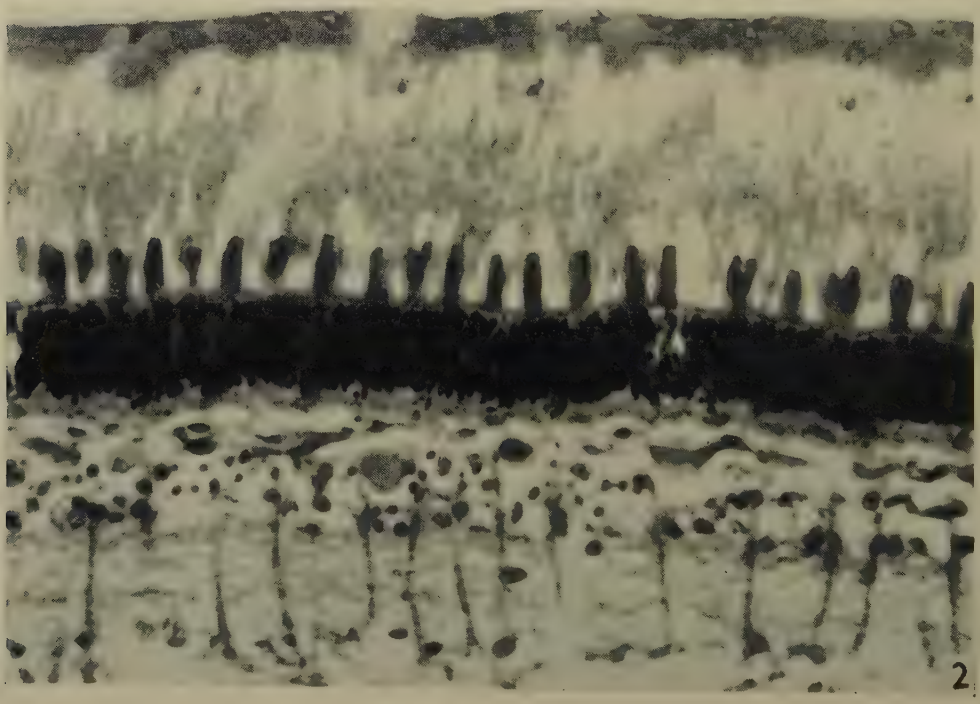
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NOTES ON THE MODE OF LIFE OF THE TELLINIDAE (LAMELLIBRANCHIA)

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(Text-figs. 1–3)

Ten species of Tellinidae occur in British waters, belonging to the genera *Arcopagia*, *Tellina*, *Gastrana* and *Macoma* (Winckworth, 1932, 1951). This paper is concerned only with *Arcopagia crassa*, and the commoner species of *Tellina*: *T. squalida*, *T. tenuis*, *T. fabula*, *T. donacina* and *T. pygmaea*. These species burrow in sands, muds or gravels, maintaining connexion with the overlying water through their long and extensile siphons. Some account of the habitat of individual species is given by Yonge (1949).

The majority of burrowing lamellibranchs occupy a more-or-less vertical position when buried, but a few (e.g. *Cochlodesma*, see Allen, 1958) lie horizontally beneath the surface. While studying dispersal in a population of *T. tenuis* (Holme, 1950), it was noticed that the majority of specimens excavated at low tide lay on their sides, but it was not at the time appreciated that they always lay with one side, the right, uppermost. Yonge (1949) assumed that members of the Tellinidae occupy a vertical position when buried, but he is of the opinion (personal communication) that *T. tenuis* makes considerable vertical movements in the sand according to the state of the tide, so that the position in which it is found at low tide may be rather different from that when covered by water.

Both MacGinitie (1935) and Fitch (1953) record that the North American shore clam *Macoma nasuta* lies on its left side, the posterior end of the shell, which is curved to the right, being inclined upwards, so that it seemed possible that other members of the Tellinidae might show similar habits.

Observations on the habits of the Tellinidae have been made as follows: (*a*) on the position of animals dug up at low tide on the shore, (*b*) on the method of burrowing, (*c*) on the shape of the valves.

POSITION OF ANIMALS ON THE SHORE

The only species at all common on the shore are *Tellina tenuis*, *T. fabula*, and *T. squalida* (in south-west England). Careful excavation, or the chance exposure of a specimen when digging, has shown that all three species lie on their left sides, usually within about 30° of the horizontal. Of twenty-two specimens of *T. tenuis* excavated in the Exe estuary, twenty-one lay on the

left side, and one on its right side, but fewer observations have been made on the other two species, which were, however, invariably found on their left sides.

The normal habit of these species is shown in Fig. 1, but it should be noted that the disposition of the siphons is not based on any new observations. Yonge (1949) describes the long inhalant siphon of *T. tenuis* the tip of which lies along the surface of the sand. He figures the exhalant siphon as just projecting above the surface, but states that 'the exhalant siphon was never observed to project above the sand although it must be continually open to allow the through passage of large quantities of water which enter the other siphon'.

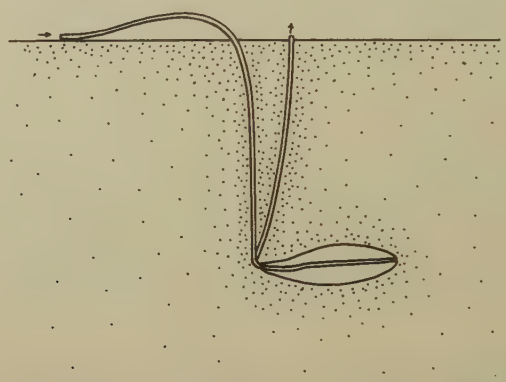

Fig. 1. Diagram of *Tellina squalida* lying on its left side in the sand. The disposition of the siphons is hypothetical, see text. × ½.

In *Arcopagia crassa*, the only species I have observed in detail, the exhalant siphon has never been seen at the surface, and the inhalant siphon tends to project upwards rather than lie along the surface. If such observations in the laboratory are a guide to the natural habit, they suggest that the normal position of the siphons in other Tellinidae requires further observation. Possibly *Arcopagia* disposes its inhalant siphon so as to filter as a suspension rather than a deposit feeder, and it may be that the exhalant siphon is not normally extended to the surface. The gravel in which it lives is quite permeable, so that an exhalant stream could be discharged well below the surface of the gravel. This recalls the habit of *Macoma nasuta* described by MacGinitie (1935) and figured in MacGinitie & MacGinitie (1949).

OBSERVATIONS ON BURROWING UNDER WATER

Arcopagia crassa. Specimens were placed on the surface of shell gravel and their burrowing observed. Specimens lying on their left side on the surface (the normal position when buried) would after a time cautiously

extend the triangular tongue-like foot, which was then directed downwards into the gravel (Fig. 2). Burrowing movements follow the normal pattern, the foot being extended and then thickened and contracted to draw the shell into the substratum. When a grip was obtained in the gravel the posterior end of the shell was raised by successive contractions to about 45° to the horizontal, and the animal then burrowed obliquely into the gravel at this angle, the ligament axis being approximately horizontal (Fig. 3).

Specimens lying on their right side started to burrow in the same way, but successive contractions of the foot raised the shell up to and just beyond the vertical, the shell being partially turned over before it entered the gravel. Subsequent contractions of the foot continued the turning of the shell, so that it finally disappeared below the surface at an angle of about 45°, with the right side uppermost. Of several dozen specimens allowed to burrow and subsequently excavated, not one had its left valve uppermost. While a few were nearly vertical, the majority, particularly those 7 cm or more below the surface, had the right valve uppermost.

The shell of *Arcopagia crassa* is rather heavy, and some effort is required to turn it over. In *T. tenuis*, *T. squalida* and *T. donacina* the animal is smaller and the shell lighter and thinner. In these the shell, of those placed on their right sides, was not raised beyond the vertical before it entered the substratum. Successive burrowing movements turned the shell over while it was entering the soil so that when it disappeared from sight it might have only just reached the vertical position. Specimens placed on their left sides burrowed obliquely, as in *Arcopagia*. Observation of these species was made on the shore, and, when specimens were dug up a few minutes later, the right side was always uppermost. Only one or two specimens of *T. donacina* were observed, but quite a number of observations were made on the other two species.

THE SHAPE OF THE VALVES

The majority of the Tellinidae shows some asymmetry between the two valves, and in *T. fabula* the right valve exhibits oblique striae which are absent in the left valve. In some the posterior ends are inclined to one side: a marked inclination to the right is shown in the American *Macoma nasuta*, and the same tendency is shown, to a lesser degree, in some British species. In *T. squalida* the posterior end is somewhat produced, and both valves show a slight though distinct curvature to the right (Fig. 1), and a similar but lesser curvature is shown in *T. donacina*, *T. fabula* and *T. tenuis*. In *Arcopagia crassa* the right valve is a little deeper and more convex than the left. The posterior end, if anything, points a little to the left (Fig. 3) and there is a double fold radiating towards the postero-ventral margin of the shell. A rather similar fold occurs in *T. pygmaea*. In these two last species, therefore, the shell gives no indication of the animal's habits.

Fig. 2. *Arcopagia crassa*, showing burrowing movements of foot. One siphon is just protruding from the shell. $\times \frac{1}{2}$.

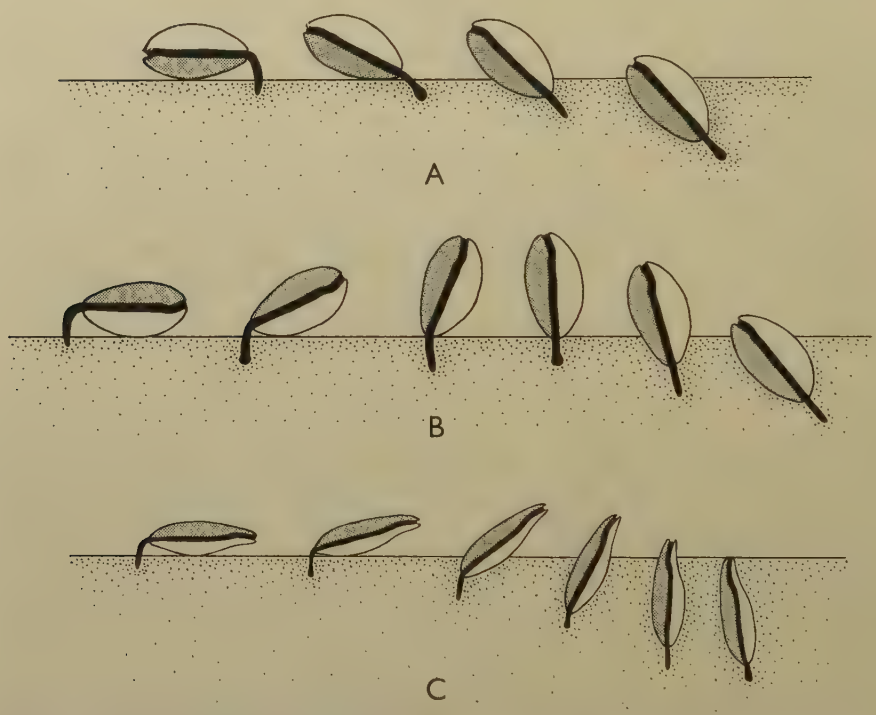

Fig. 3. Burrowing of tellinids, viewed from the ventral side. The left valve is stippled in each instance. $\times \frac{2}{3}$. A. *Arcopagia crassa* placed on its left side burrows obliquely at about 45°. B. *A. crassa* placed on its right side turns over before entering the sand. C. *Tellina squalida* placed on its right side turns over as it burrows down into the sand. Placed on its left side it burrows as in *Arcopagia crassa* (A).

There does not appear to be any asymmetry of the internal organs connected with the mode of life.

The habit of lying on one side would increase the ease with which a burrowing bivalve could make horizontal migrations. In deposit-feeding forms such as the Tellinidae this might allow a fuller exploitation of the soil surface by the siphons through the dispersal of individuals away from one another. In *Tellina tenuis* populations on the Exe such movements result in a very evenly distributed or 'overdispersed' population (Holme, 1950); alternatively the horizontal migrations may be of use in finding more favourable areas or for migrations up and down the shore.

SUMMARY

Evidence from animals dug up on the shore, from observations on burrowing, or from shell shape indicates that the following species normally lie below the surface with their right valve uppermost: *Tellina squalida*, *T. tenuis*, *T. fabula*, *T. donacina*, and *Arcopagia crassa*. Information on the habits of the remaining Tellinidae has not been obtained.

Animals placed on the surface of the soil with the left side up turn themselves over as they burrow. In *Arcopagia* the shell is turned over beyond the vertical before the animal enters the substratum, but in the remaining species studied the shell is gradually turned over so that it disappears below the surface almost vertically and subsequently completes the turning movement beneath the surface.

The disposition of the siphons of *Arcopagia* may differ somewhat from that of *T. tenuis* as described by Yonge, and there is a need for further observation on the siphons of the other species.

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SHELL FORM IN *VENERUPIS RHOMBOIDES*

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(Plate 1 and Text-figs. 1–9)

Venerupis rhomboides (Pennant) is one of the commonest and most widely distributed lamellibranchs on the bed of the English Channel, where it burrows in deposits of gravel or coarse sand from low-tide mark to 80 m or more. It ranges southward to the Mediterranean, but does not occur far north of the British Isles (Madsen, 1949, as *Tapes edulis* (Chemnitz)).

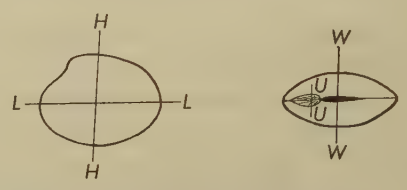
Populations collected in different localities and at different depths off Plymouth showed considerable variations in shape and shell-proportions, to the extent that I at one time considered that two species occurred (Marine Biological Association, 1957, p. 330)—a broader and more solid form corresponding to the *Venus sarniensis* of Turton (1822), and a more slender form corresponding to the typical *Venus rhomboides* described by Pennant (1777). Bucquoy, Dautzenberg & Dollfus (1893) illustrate a number of varieties of *Venerupis rhomboides* from different localities, but even in a single one of my samples forms corresponding to several of these varieties sometimes occur. No differences in the siphons or other soft parts have been described, and I have been unable to find any significant differences in these structures.

At Plymouth a typical *rhomboides* population occurs in the Eddystone shell-gravel at *ca.* 45 m, and a typical *sarniensis* population at the mouth of the River Yealm, where occasional living specimens and many empty shells presumed to have been washed in from the river channel may be found.

The differences between the two forms may be summarized as: (1), the shell of *rhomboides* is more slender and is narrower across the two valves (these differences are expressed quantitatively in Table 1); (2), the lunule is narrow and lanceolate in *rhomboides*, broader and cordiform in *sarniensis*; (3), the colour-patterning of the shell in *rhomboides*, which is of shades of pink or reddish brown on a whitish or sometimes ferruginous background is described in the literature: *sarniensis* has similar markings, but with a tendency towards a more purple colour-pattern in some individuals. Although these differences may appear trivial, the range of form is such as to indicate the possibility of the existence of two species. The *sarniensis* form is at first sight so different from the typical form as to be sometimes confused with *V. aurea*.

METHODS

Comparisons of the shell-proportions of different populations were based on the following measurements (Text-fig. 1) made with vernier callipers: Length: the greatest antero-posterior measurement. Height: the smallest calliper reading in the dorsi-ventral plane through which the shell will pass. This axis is not quite at right angles to the long axis, and therefore differs slightly from the height as usually measured. Width: the greatest measurement



Text-fig. 1. Measurements made on *Venerupis rhomboides* shells, lateral and dorsal views. *L*, length; *H*, height; *W*, width; *U*, lunule width.

in a lateral plane across a pair of closed valves (measurements of single valves are unsatisfactory as the two valves often differ slightly in depth). Lunule width: the width of the lunule at its widest point, measured with dividers. Such measurements are only a crude means of defining the characteristics of shells which differ in shape as well as in proportions, but a selection of representative shells is shown in Plate I.

Depths are from the echo-sounder, corrected so far as possible for tidal height. Since many of the samples are from anchor-dredge hauls in which the

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EXPLANATION OF PLATE I

Fig. 1. Representative right valves of *V. rhomboides* from populations living at different depths. *ca.* × 0·75. 4–5 cm length group. *H*/*L*: height/length ratio.

A. One of the more slender shells in the Eddystone shell gravel population. *H*/*L* 0·637. Depth 45 m.

B. Representing the median ratio of the Eddystone shell gravel population. *H*/*L* 0·658.

C. Station DS 5. *H*/*L* 0·667. Depth 18 m.

D. Representing the median ratio of the Plymouth Sound population. *H*/*L* 0·691. Depth 6·5 m.

E. Representing the median ratio of the Yealm population. *H*/*L* 0·720. Depth 2·5 m.

F. Salcombe. *H*/*L* 0·780. Depth 3·5 m.

Fig. 2. Antero-dorsal view of lunule and umbo. About natural size. The lunule is the lanceolate or cordiform area just below the umbo. Its outline has been inked in on one side in two specimens. 4–5 cm length group. *U*/*L*: lunule width/length (of shell) ratio.

A. Eddystone shell gravel. *U*/*L* 0·067. Depth 45 m.

B. DS 57. *U*/*L* 0·073. Depth 25 m.

C. Yealm. *U*/*L* 0·096. Depth 2·5 m.

D. Salcombe. *U*/*L* 0·134. Depth 3·5 m.

Fig. 1

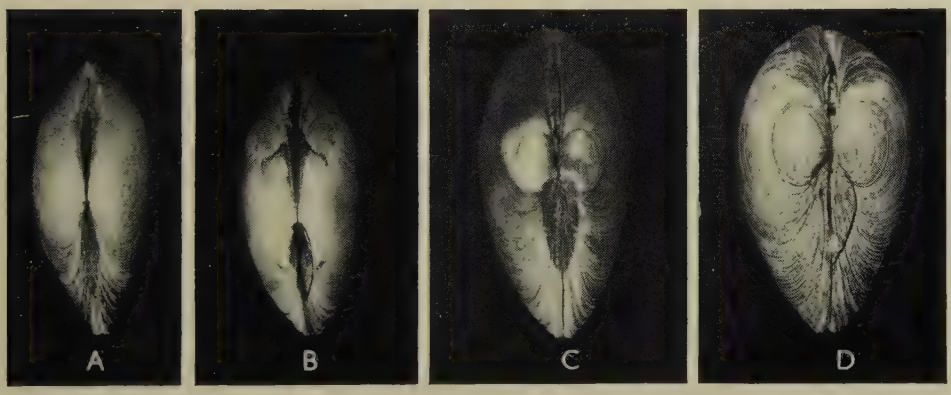


Fig. 2

(*Facing p.* 706)

ship is not directly above the sampling gear, a small error may be present in some of the soundings.

The few samples from the shore at the Yealm and at Salcombe were mainly of dead shells, but included occasional living specimens burrowing in the deposit near low-water mark. Clearly the main population lay offshore, and an estimated depth was made equal to one-half of the maximum in the adjoining river channel.

It will be appreciated that a potential source of error exists in many of the depths quoted, but these are not great enough to mask the correlation of shell form with depth described later in this paper.

RESULTS

COMPARISON OF REPRESENTATIVE POPULATIONS

Shell-proportions of the Yealm and Eddystone populations together with those from a position in Plymouth Sound are shown in Table 1. As there is some change in proportions with growth, the means for different cm groups have been calculated separately. Changes in height and width ratios appear to

TABLE 1. MEAN SHELL-PROPORTIONS FOR THREE REPRESENTATIVE POPULATIONS OFF PLYMOUTH

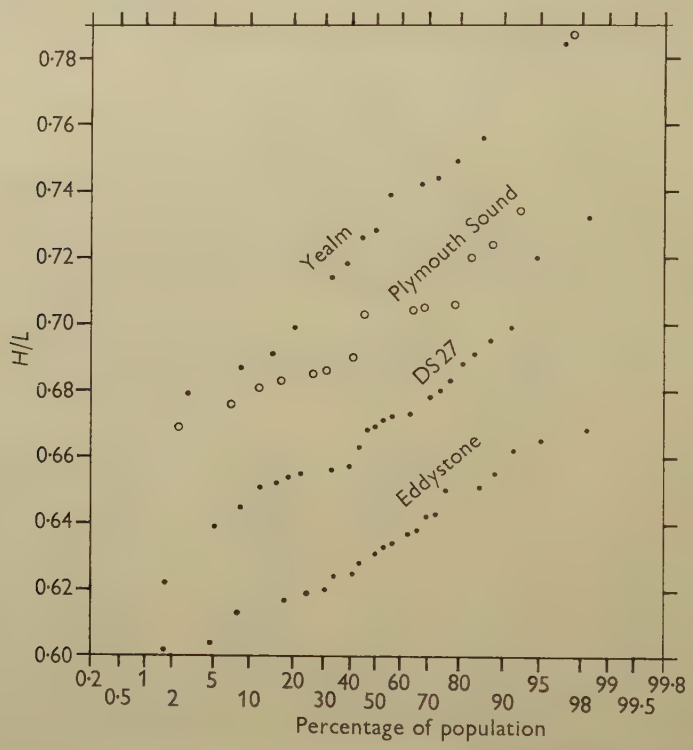
	Length class (cm)	No. in sample	H/L	W/L	U/L
Yealm (depth 2·5 m)	3–4	17	0·728	0·464	0·110
	4–5	100	0·723	0·472	0·118
	5–6	11	0·729	0·486	0·119
Plymouth Sound (depth 6·5 m)	4–5	90	0·698	0·441	0·100
Eddystone shell gravel (depth 45 m)	3–4	31	0·633	0·378	0·075
	4–5	34	0·666	0·411	0·072
	5–6	21	0·674	0·424	0·075

H, height; *W*, width; *L*, length; *U*, lunule width. For convenience, the length classes have been quoted to the nearest whole number; e.g. the 3–4 cm length group includes shells between 3·00 and 3·99 cm length.

be more marked in the Eddystone than in the Yealm populations. It will be noted that in each instance the Plymouth Sound ratios are intermediate between those of the Eddystone and Yealm populations, and after a number of other populations had been measured it was found that height, width and lunule measurements all followed similar trends, so that a single ratio, height/length, was considered sufficient to characterize a population. Subsequent measurements have been restricted to this ratio, and the median rather than the mean value has been employed as being less affected by occasional specimens with particularly high ratios, as occur in some shallow-water samples.

COMPOSITION OF THE POPULATIONS

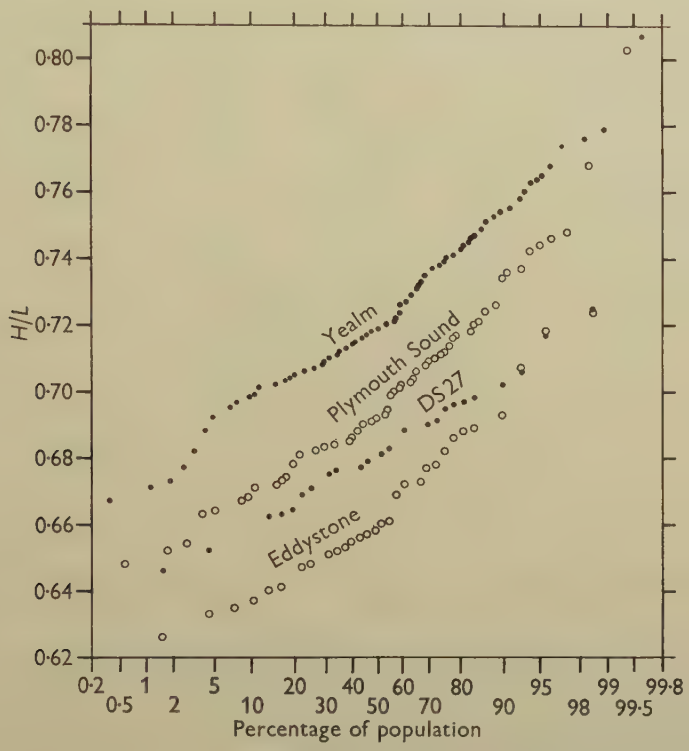
If a cumulative plot of height/length ratios is made on arithmetic probability paper, some information on the composition of the populations may be obtained. A population of a single species would be expected to give a straight-line plot, and a population containing two species or forms with different median ratios would give a plot with an inflexion corresponding to the transition between the two forms. Text-figs. 2 and 3 show the Eddystone, Yealm



Text-fig. 2. Cumulative plots of height/length ratio on arithmetic probability paper, 3–4 cm length class.

and Plymouth Sound populations, together with another intermediate population from Poole Bay (DS 27), plotted on probability paper. None of the plots gives a very good straight line, but the small inflexions may be due to length-grouping of individuals. Individuals (whether living or dead) tended to be aggregated into length-groups, corresponding perhaps to different year-classes. Since proportions change slightly with growth this grouping would tend to produce small inflexions in the plots. The two intermediate populations do not appear to be bimodal, but any such tendency might be masked to some

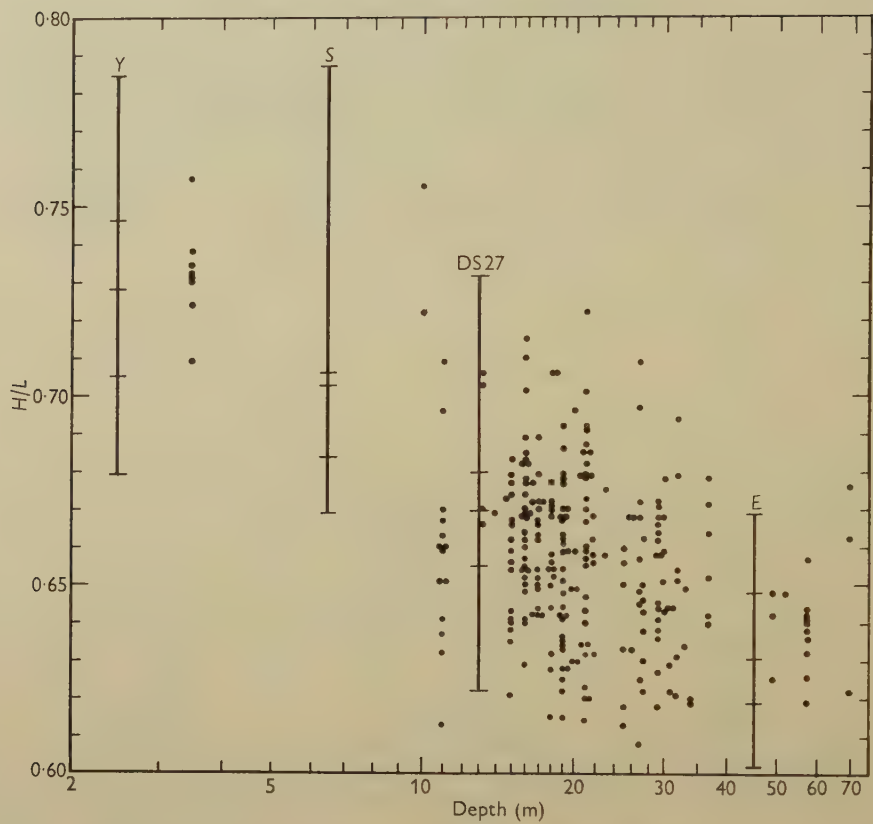
extent by this length-grouping of the populations. However, not only the medians but also the extreme values for each population tend to form a series which makes it seem unlikely that the intermediate populations are mixtures of the two extreme forms. The most likely explanation appears to be that the differing shell-proportions are an expression of variation within a single species.



Text-fig. 3. Cumulative plots of height/length ratio on arithmetic probability paper, 4–5 cm length class.

OCCURRENCE OF THE DIFFERENT FORMS

There appeared to be some relation between shell proportion and depth of water, and accordingly samples from different areas, mainly in the English Channel, were measured and the height/length ratio plotted against depth. The plots are conveniently made on a log-depth scale, when something approaching a straight-line relationship is obtained. Samples usually contained some living individuals, but the bulk of each sample was of dead shells. Scatter diagrams for the two length-groups are shown in Text-figs. 4 and 5. There appears to be a continuous series between the broad shallow-water form and the more slender deeper-water form, suggesting again that only one species is

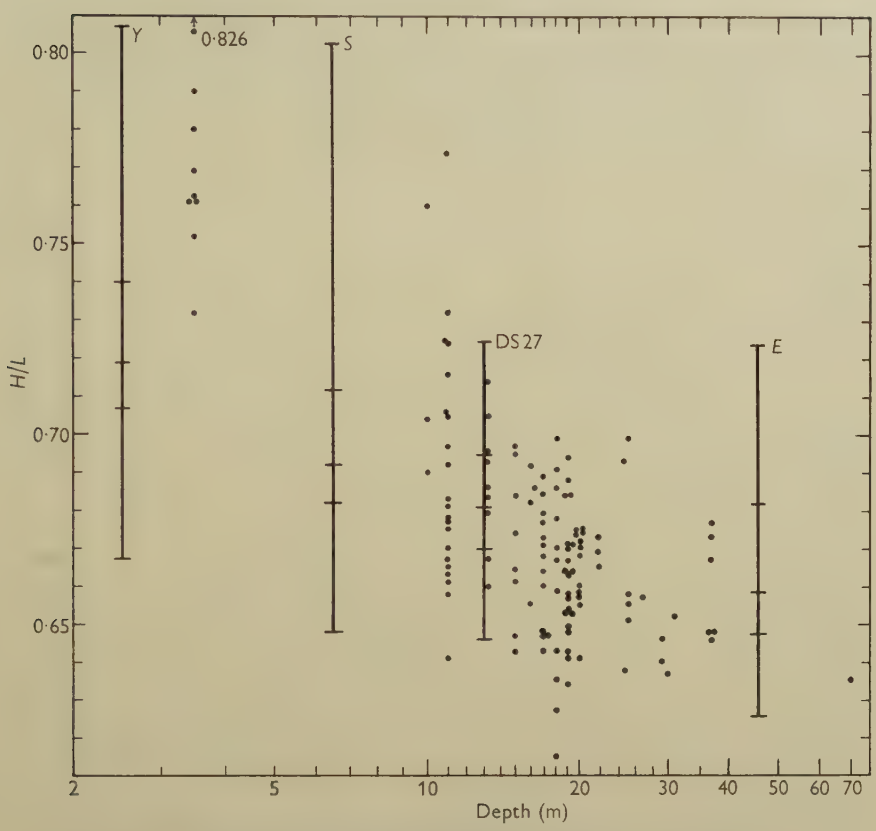


Text-fig. 4. Scatter diagrams of height/length ratios of different samples plotted against depth on a log scale, 3–4 cm length class. The points represent individual specimens, but for the four larger samples, from the Yealm, Plymouth Sound, DS 27, and Eddystone, the limits, medians and upper and lower quartiles are shown.

present. The slope of the plot is steeper for the 3–4 cm than for the 4–5 cm group. In the former there is no overlap between the ratios of individuals from the Yealm and the Eddystone, but, owing to changing proportions with growth, differences are less marked in the 4–5 cm group.

USE OF SHELLS AS INDICATORS OF DEPTH

Whatever the biological reasons for the change in shell proportions, the correlation with depth is apparent. It follows that it should be possible to use shells as indicators of depth, and by this means obtain information on movements of shells along the sea floor, the original habitat of shells washed up on a beach, or of specimens contained in shell deposits formed perhaps when the sea was at a different level.



Text-fig. 5. Height/length ratios plotted against depth, 4–5 cm length class. For explanation see legend to Text-fig. 4.

In Text-figs. 6 and 7 are plotted the median height/length ratios for samples of five or more individuals in each length group (see Appendix I, Table 4). In spite of the small size of some samples, the points approximate to a straight line, showing a negative correlation between the shell ratio and (log) depth. The regression equations for median values are:

3–4 *cm group*

$$Y = 0{\cdot}755 - \frac{\log X \times 0{\cdot}00056}{0{\cdot}007525},$$

$$\log X = (1142{\cdot}54 - 1476{\cdot}3\,Y)\,(0{\cdot}007525).$$

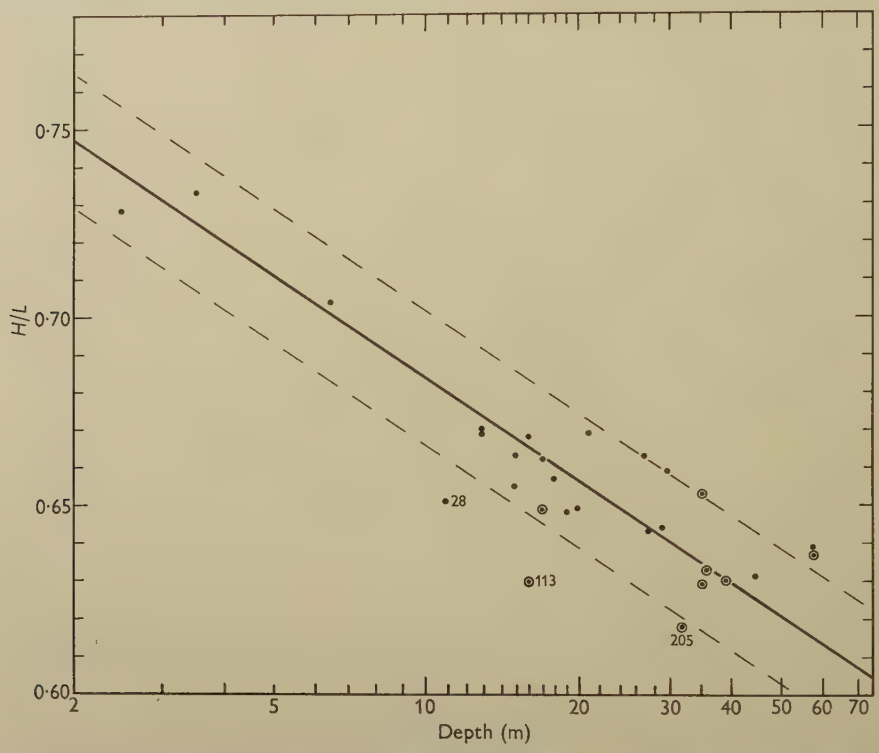
4–5 *cm group*

$$Y = 0{\cdot}737 - \frac{\log X \times 0{\cdot}000395}{0{\cdot}007525},$$

$$\log X = (1607{\cdot}23 - 2147{\cdot}8\,Y)\,(0{\cdot}007525),$$

where X is depth in metres, and Y the H/L ratio.

(The equations are represented in this form to allow direct plotting on arithmetic graph paper. On the X axis 40 squares = 2 m, 80 = 4 m, 120 = 8 m, etc., the relationship between the graph scale X_1 and the depth being given by $X_1 = \log X/0{\cdot}007525$.)



Text-fig. 6. Regression of depth on median height/length values, 3–4 cm length class. ●, samples used in plotting regression; ⊙, samples used in depth estimation. Some station numbers are included. The two broken lines are plots corresponding to twice the Standard Error. For explanation see text.

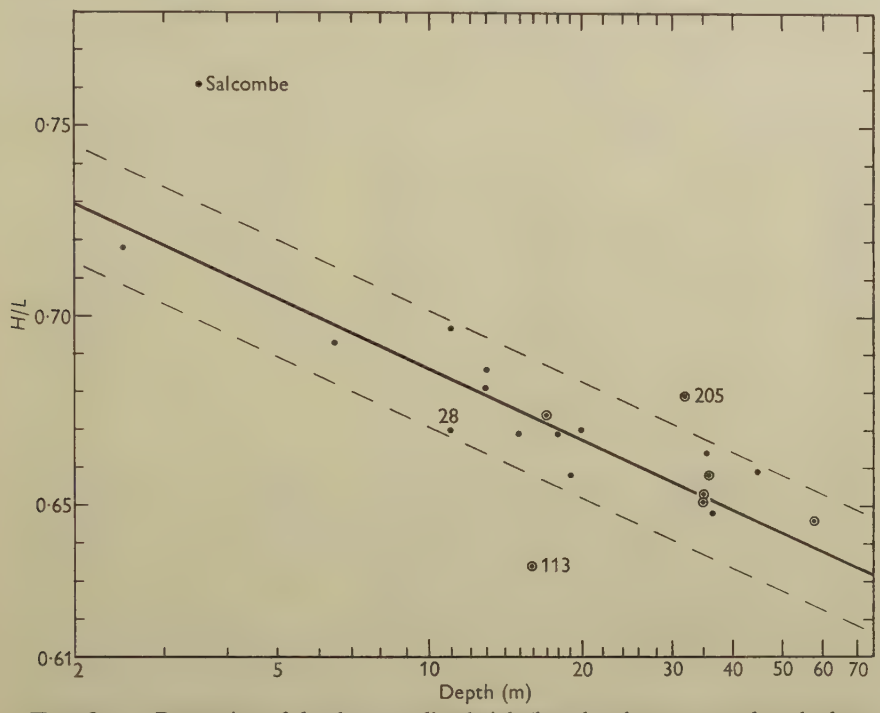
In the 4–5 cm group the Salcombe sample has been excluded as it clearly deviates from the relationship shown by the other points. This could be due to an error of only a metre or two in the estimated depth, but the Salcombe population appears to be broader than any found elsewhere. It was later suggested that the regression equations should be 'weighted' according to the numbers in each sample. The equations were therefore re-calculated, each sample being weighted according to the square root of numbers in the sample.

For the 3–4 cm group the equations then become

$$Y = 0{\cdot}756 - \frac{\log X \times 0{\cdot}000567}{0{\cdot}007525},$$

$$\log X = (1149{\cdot}14 - 1485{\cdot}9\,Y)\,(0{\cdot}007525).$$

The regression lines plotted from these equations do not differ sufficiently from those from the 'unweighted' results to justify any corrections, and further calculations were all based on unweighted values.



Text-fig. 7. Regression of depth on median height/length values, 4–5 cm length class. For explanation see legend to Text-fig. 6.

The correlation coefficients (r) are $-0{\cdot}909$ (3–4 cm) and $-0{\cdot}921$ (4–5 cm), with t values of 12·74 and 7·83 respectively, showing a highly significant ($P = < 0{\cdot}01$) negative correlation between H/L and depth.

Standard errors, on the basis of the arithmetic scale noted above, are:

3–4 *cm* $S_{x1} = 12{\cdot}96, \quad S_y = 0{\cdot}00798.$

4–5 *cm* $S_{x1} = 16{\cdot}58, \quad S_y = 0{\cdot}00711.$

To estimate depth from a given sample, the regression line of X on Y is plotted, and parallel lines are plotted at a distance of twice the standard error (S_{x1}) on either side of it. The probability of the median value lying within these limits is about 0·05.

Equations for plotting lines corresponding to twice the standard error are approximately:

3–4 *cm* $\log X = (1142{\cdot}54 - 1476{\cdot}3\,Y \pm 26)\,(0{\cdot}007525).$

4–5 *cm* $\log X = (1607{\cdot}23 - 2147{\cdot}8\,Y \pm 33)\,(0{\cdot}007525).$

The estimated depth and probable depth range of a sample may then be calculated, or read off the graphs. Representative values are shown in Table 2, but estimated depth ranges exceeding 65 m should be treated with reserve.

TABLE 2. ESTIMATED DEPTH AND PROBABLE DEPTH RANGE FOR *H/L* MEDIAN VALUES IN THE 3–4 AND 4–5 CM GROUPS

Depths, in metres, to nearest ½ m

	3–4 cm			4–5 cm	
H/L (median)	Estimated depth	Probable range	*H/L* (median)	Estimated depth	Probable range
0·740	2·5	1·5– 3·5	0·730	2·0	1·0– 3·5
0·710	5·0	3·5– 8·0	0·710	4·0	2·5– 7·5
0·700	6·5	4·0– 10·5	0·700	6·0	3·5– 10·5
0·690	8·5	5·5– 13·5	0·690	9·0	5·0– 15·5
0·680	11·0	7·0– 17·5	0·680	12·5	7·0– 22·5
0·670	14·5	9·0– 22·5	0·670	18·5	10·5– 32·5
0·660	18·5	12·0– 29·0	0·660	26·5	15·0– 47·5
0·650	24·0	15·0– 37·5	0·650	39·0	22·0– 68·5
0·640	31·0	19·5– 48·0	0·640	56·5	32·0–100·0
0·630	39·5	25·5– 62·0			
0·620	51·5	32·5– 80·5			
0·610	66·0	42·0–104·0			

Eight samples, almost entirely of dead shells, which were neither included in the data for plotting regressions nor in the scatter diagrams in Text-figs. 4 and 5 are listed in Table 3. These have been used as a check on the accuracy of depth estimations. Stations 116, 133, 135, 137 and 206 show a reasonably good agreement with the recorded depths. At station 112 there is some under-estimation of depth, but the samples at 113 show a wide discrepancy from the recorded depth. The lowest *H/L* ratio in the 3–4 cm class was 0·616 and the lower ratio in the 4–5 cm class was 0·619. Referring to Text-figs. 4 and 5 it is clear that such low ratios would not be expected at depths less than about 17 and 26 m respectively. It therefore seems unlikely that these shells were living *in situ*. Station 113 was in Whitsand Bay, and the chart shows depths ranging from 11 to 38 m within about half a mile of this position. Assuming that the sampling depth was correctly recorded, it seems likely that the dead shells had been washed in from deeper water in the vicinity.

Station 205 also shows large discrepancies from the recorded depth. This sample, off the Normandy coast, was about 5 miles south of 206, in which a larger sample gave good agreement with depth. Re-examination of the sample showed that the dead shells could be divided into two categories: those fresh and in good condition, and old shells bored by *Cliona*, etc. There were also some shells in the 5–6 cm group, and when thus separated the individual ratios were:

Fresh shells—3–4 cm *H/L*: 0·603, 0·618, 0·624. 4–5 cm *H/L*: 0·629, 0·660. These indicate depths of *ca.* 54 and 45 m respectively.

Old shells—4–5 cm *H/L*: 0·678, 0·679, 0·682, 0·682, 0·697. 5–6 cm *H/L*: 0·656, 0·657, 0·676, 0·682, 0·697. The 4–5 cm group indicates a depth of

about 11·5 m (probable range 7–20·5 m) and the 5–6 cm group has ratios of the same order.

It is reasonable to suppose that the sample contains two distinct populations, distinguished both by the condition of the shells and by their H/L ratios. The recorded depth of 32 m is well below that indicated by the fresh shells, but their number is small and they could well have been from a resident population. The old shells are clearly from a shallow-water population, either washed out from near the coast some twelve miles to the south or derived from an old shell deposit formed in shallow water.

TABLE 3. ESTIMATION OF DEPTH FROM MEDIAN H/L RATIO

Depths in metres

Station	Length group (cm)	No. in sample	H/L (median)	Estimated depth	Probable depth range	Recorded depth
DS 112	3–4	5	0·637	33·0	21 –52	58
	4–5	4	0·646	45·0	25·5–80	
DS 113*	3–4	6	0·630	39·5	25 –62	16
	4–5	2	0·634	70·5	>39·5	
DS 116	4–5	3	0·653	34·5	19·5–61·5	35
DS 133	3–4	7	0·630	39·5	25 –62	39
DS 135	3–4	4	0·629	40·5	26 –64	35
	4–5	8	0·651	37·5	21 –66	
DS 137	3–4	5	0·649	24·5	15·5–38·5	17
	4–5	14	0·674	16·0	9 –28	
DS 205*	3–4	3	0·618	54·0	>34·5	32
	4–5	7	0·679	13·0	7 –22·5	
DS 206	3–4	22	0·633	37·0	23 –55	36
	4–5	8	0·658	28·0	16 –50	

* Samples showing a wide discrepancy between estimated and recorded depth.
The quoted median of samples of even number is the mean of the two values adjacent to the mid-point of the series.

One other sample, DS 28, which was used in plotting the regressions, also appears to show evidence of transport along the bottom. Only living shells were measured, and these gave appreciably lower shell ratios in relation to depth than other samples from Poole Bay. The 3–4 cm group gives an estimated depth of about 23 m and the 4–5 cm group an estimated depth of 18·5 m, the recorded depth being only 11 m. This sample was taken close to the tidal race off Old Harry Rocks, so that it seems probable that the specimens had been washed into shallower water while still alive.

It would appear that under favourable circumstances a close correlation of shell ratio and depth may be obtained, even with comparatively small samples (cf. Table 3). There are a number of variables and sources of error in such a comparison. These include the small size of the sample and consequent sources of inaccuracy in determining a true median, the possibility that shells have been transported along the sea floor from a different depth, inaccuracies in determining or estimating the depth of the samples on which the regressions

are based, and inherent variability in different populations. Some of the estimates in Table 3 based on small samples are so close to the recorded depths as perhaps to be fortuitous. The fact that a relatively good correlation has been obtained in spite of the sources of variation mentioned above suggests that the correlation is in fact much closer than the stated limits indicate.

DISCUSSION

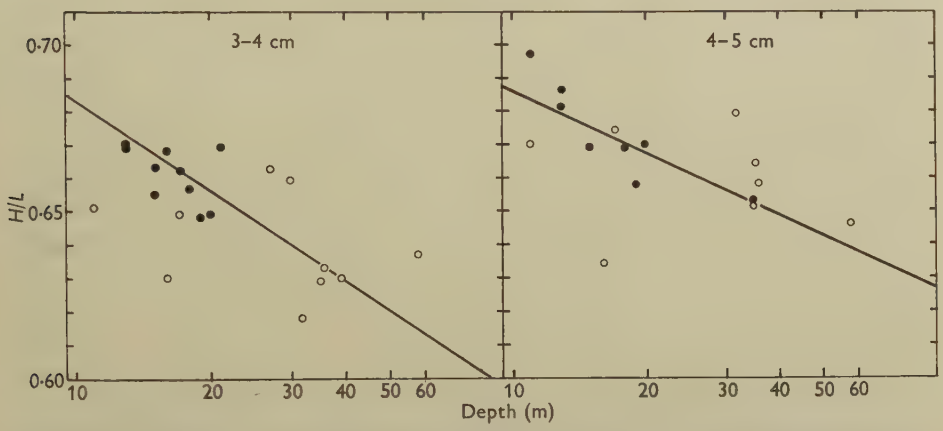
Although it is well known that the form of some molluscs, as of other organisms, varies in different habitats, there is little information on the causes of such variation. The shell-shape and proportions of fresh-water lamellibranchs have been studied by a number of workers (see Eagar, 1948, for references), and shell form has been related to such factors as water-movement, wave-action, or the nature of the bottom, and there are also characteristic differences between the form in stream as opposed to lake environments.

Ohba (1956) has studied the effects of varying population density on growth, under experimental conditions, of the marine lamellibranch *Venerupis semidecussata*. At high densities not only was growth (measured as increase in weight) retarded, but increase in length was proportionately less than that in height and width of the shell. In this instance shell proportions may well have been influenced by the rate of growth. In *V. rhomboides* the question of crowding does not occur, at any rate within individuals of the same species, but it may be that other factors produce individuals of differing shell proportions through their effect on the rate of growth.

In *Venerupis rhomboides* there appears to be a correlation of shell form with depth of water, but as many other factors change with depth it is possible that the observed variation may be due to some other cause.

Since some workers have found shell-shape to be partly related to the type of deposit, a brief survey was made of the range of sediments inhabited by *V. rhomboides*. This species on the whole occurs only in deposits containing some gravel or coarse sand, but the proportion of mud (silt plus clay) appears to vary considerably. No sediment analyses have been made, but the deposits have been classified as 'clean' or muddy. Taking the DS series of samples the median plots have been distinguished on this basis (Text-fig. 8). It will be seen that on the whole the shallow-water offshore samples (10–20 m) tend to be more muddy than the deeper samples. This might, however, be expected from any random collection of bottom samples taken at varying depths. The usual sequence of deposits is a belt of clean sand or shingle on the shore and extending seawards to only a few metres depth; beyond this deposits may be of sand or gravel, containing a variable and sometimes large proportion of mud, giving way at greater depths to fairly clean sands or gravels. Clean gravel deposits are almost invariably subject to considerable scour, and on the shore and in shallow water may be almost barren, but in deeper water, such as

at the Eddystone, the scour is evidently insufficient to prevent the establishment of a burrowing fauna. Muddy deposits in shallow water would be associated with less bottom disturbance, and it seems possible that in shallower water a species like *Venerupis rhomboides* can only establish itself in the more stable gravel deposits which at the same time permit deposition of mud. The only way of determining whether depth or soil grade is responsible for the shell proportions of *V. rhomboides* would be to obtain samples either from a deep-water muddy gravel or a clean inshore gravel. Deep-water muddy gravels are scarce in the Channel, but there are places on the shore where the



Text-fig. 8. Regressions of depth on median height/length values. Only DS stations plotted; regression lines as in Figs. 6 and 7. ●, samples from muddy sediments; ○, samples from 'clean' sediments.

deposit is clean and coarse, and the bottom not too disturbed. One such place is the Scilly Isles where there is a coarse granite sand between some of the Islands. *Venerupis* is unfortunately rare there, and some of the empty shells collected may have been washed in from similar deposits in shallow water offshore. Five specimens, all over 5 cm long, had height/length ratios between 0·731 and 0·752, clearly corresponding to the broad shallow-water form. Similarly, the type locality of Turton's *Venus sarniensis* is from low-tide mark in sand at Guernsey, where the sands, although finer grained than at Scilly, would be unlikely to be very muddy. It therefore seems unlikely that the nature of the deposit is responsible for the observed variation in shell ratio.

Temperature is another factor which changes with depth. In the western half of the English Channel a thermocline becomes established in the summer months, so that off Plymouth there is a difference of about 3° between surface and bottom temperatures, and one may therefore expect a corresponding gradient of bottom temperatures according to depth. However, the samples were taken over a wide area of the Channel, with corresponding differences in

bottom temperature, and even in the eastern Channel, where there is no vertical temperature gradient even in summer, a good correlation of shell shape with depth was shown by the few samples from this area. It therefore seems unlikely that shell form is correlated with temperature.

Although some vertical stratification of food, whether plankton or detritus, must occur, it seems unlikely that this would be so precisely zoned by depth, especially in areas where vertical mixing occurs, as to account for the observed changes in the shell.

One factor which is invariably correlated with depth is light penetration. However there are very considerable variations in penetration in different parts of the Channel (cf. Gall, 1949), so that the correlation of shell form with depth could hardly be attributed to this factor.

It would appear that no factor other than pressure is consistently related with depth, and we must therefore conclude that this is the most likely cause of the change in shell proportions. It seems unlikely that any chemical reactions would be significantly changed over the range of pressures involved (say 1 to 7 atmospheres), but there remains the possibility that the animals are sensitive to and respond to these relatively small differences in pressure. Digby (1961) cites several instances of invertebrates sensitive to hydrostatic pressure changes, and recent work by W. R. Lowenstein (1960) has shown that a species of dogfish is sensitive to pressure changes corresponding to differences in depth of a metre or so, so that it is possible that other animals are more susceptible to pressure differences than is at present supposed.

If this hypothesis is rejected we must seek an answer in terms of an interrelationship of the stability and grade of bottom deposit with current and/or wave-disturbance, or in the food supply.

I am indebted to the captains and crews of the Laboratory's research vessels for much of the material used in this work, and to Dr A. D. Ansell and Dr N. S. Jones for samples from the Clyde and Isle of Man respectively. I would also like to thank Mr G. M. Spooner for his assistance in obtaining specimens, and for reading the manuscript.

SUMMARY

Two forms of *Venerupis rhomboides* occur in Plymouth waters. A more slender form, corresponding to that described by Pennant, occurs in the Eddystone shell gravel, and a more robust form, corresponding to the *Venus sarniensis* of Turton, occurs at the mouth of the River Yealm. The two forms differ in ratios of shell height, width and lunule width relative to shell length. A population with intermediate ratios is found at 6·5 m in Plymouth Sound.

Plots of height/length ratios of these and another intermediate sample on probability paper did not indicate that the intermediate populations were

composed of mixtures of the two extreme forms, and it was provisionally concluded that these were all variants of a single species.

If the height/length ratios of series of samples from different localities are plotted against depth of water on a logarithmic scale, an inverse relationship, approaching a straight line, is obtained. Regression lines for median ratios in the 3–4 and 4–5 cm length groups have been plotted separately, as there is some change in proportion with increasing length. These show a highly significant negative correlation between shell ratios and depth.

From the regression lines it has been possible to obtain an estimate of the depth inhabited by even quite small samples of shells. Depth estimations have been made for eight samples, consisting mainly of dead shells. Six samples lay within the expected limits, and discrepancies in the other two samples are attributed to transport along the sea floor.

The possible causes of the differences in shell ratios are discussed. Previous work suggests that shell ratios may be affected by different rates of growth. Soil grade, temperature, food supply, and light penetration are considered unlikely causes of the observed differences in shell ratios, and it is suggested that the pattern of growth may be affected by pressure.

The Appendix contains a key to British species of *Venerupis*.

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APPENDIX I

TABLE 4. DETAILS OF SAMPLES USED FOR PLOTTING REGRESSION LINES (TEXT-FIGS. 6 AND 7).

The second part of the table shows additional stations, also shown in these figures, which were used for estimating depths. Samples from the Yealm, Plymouth Sound, Eddystone and Salcombe are made up of collections made on several occasions in each locality, but the remaining samples were each taken on only one occasion. 'DS' stations are from Holme (1961).

	Depth (m)	Length group (cm)	No. in sample	H/L (median)	No. of living specimens
River Yealm	2·5	3–4	17	0·728	Very few
		4–5	130	0·718	Very few
Plymouth Sound	6·5	3–4	21	0·704	Very few
		4–5	90	0·693	Very few
Eddystone shell-gravel	45	3–4	31	0·631	The majority
		4–5	34	0·659	The majority
Salcombe	3·5	3–4	8	0·733	3
		4–5	11	0·761	1
Bigbury Bay	27·5	3–4	7	0·643	All
North of Guernsey	57·5	3–4	10	0·639	Nil
Off Port St Mary, Isle of Man	36·5	4–5	5	0·648	?
East of Belle Ile, S. Brittany	29·5	3–4	9	0·644	Nil
Weymouth Bay area					
DS 5	18	3–4	12	0·657	3
		4–5	10	0·669	Nil
DS 7	19	3–4	27	0·648	1
		4–5	19	0·658	2
DS 8	27	3–4	8	0·663	Nil
DS 9	30	3–4	5	0·659	1
Poole Bay					
DS 17	13	3–4	5	0·670	4
		4–5	9	0·686	6
DS 25	21	3–4	28	0·669	19
DS 26	17	3–4	13	0·662	13
DS 27	13	3–4	29	0·669	10
		4–5	34	0·681	24
DS 28	11	3–4	10	0·651	All
		4–5	13	0·670	All
DS 29	15	3–4	6	0·663	All
DS 30	11	4–5	7	0·697	All
DS 36	15	3–4	10	0·655	All
		4–5	6	0·669	All
DS 40	16	3–4	25	0·668	All
East of Selsey Bill					
DS 58	20	3–4	6	0·649	All
		4–5	12	0·670	All
Falmouth Bay					
DS 138	?36	4–5	9	0·664	1

TABLE 4 (*cont.*)

ADDITIONAL SAMPLES (SEE TABLE 3)

	Depth (m)	Length group (cm)	No. in sample	*H*/*L* (median)	No. of living specimens
Great West Bay					
DS 112	58	3–4	5	0·637	Nil
		4–5	4	0·646	Nil
Plymouth area					
DS 113	16	3–4	6	0·630	Nil
		4–5	2	0·634	Nil
Off Fowey					
DS 116	35	4–5	3	0·653	Nil
Falmouth area					
DS 133	39	3–4	7	0·630	Nil
DS 135	35	3–4	4	0·629	Nil
		4–5	8	0·651	Nil
DS 137	17	3–4	5	0·649	Nil
		4–5	14	0·674	Nil
Off Normandy coast					
DS 205	32	3–4	3	0·618	Nil
		4–5	7	0·679	Nil
DS 206	36	3–4	22	0·633	1
		4–5	8	0·658	Nil

LOCATION OF REMAINING SAMPLES INCLUDED IN TEXT-FIGS. 4 AND 5

Number of stations, followed by total of individuals in parentheses

Mount's Bay	1 (1)	East of Selsey	8 (27)
Falmouth area	1 (1)	Rye Bay	2 (6)
Plymouth area	2 (3)	Channel Islands	1 (4)
Great West Bay	3 (5)	Quiberon area	5 (11)
Weymouth Bay area	3 (12)	Firth of Clyde	1 (3)
Poole Bay	8 (19)		

There are in addition small samples, of less than five, from certain stations used for calculating the regression lines. Only length groups containing five or more individuals were used for the calculation.

Note added in proof

Depths have been reduced to Chart Datum, which corresponds approximately to M.L.W.S.T. Probably mean tide level should have been adopted, which would have resulted in an increase in the steepness of the regression lines, since depth is plotted on a logarithmic scale. Since the relation between shell proportions and depth must be regarded as empirical and not corresponding exactly to a straight line, this small correction can be disregarded.

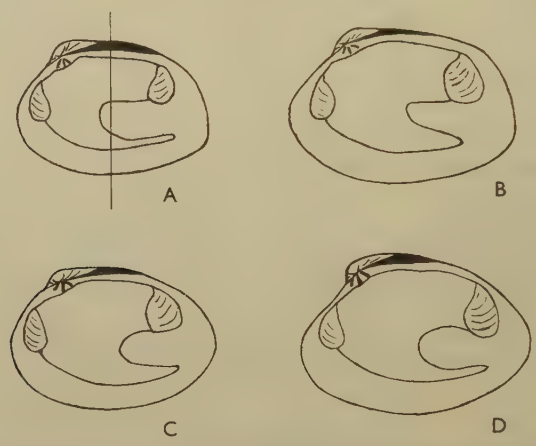
APPENDIX II

KEY TO BRITISH SPECIES OF *VENERUPIS*

Five species of *Venerupis* occur in British waters: *V. pullastra*, *saxatilis*, *decussata*, *aurea* and *rhomboides*. While the majority of specimens may be identified from figures and descriptions in the literature, there are possibilities of confusion, for example between *V. aurea* and the shallow-water form of

V. rhomboides. The following key is based on shell characters only (see also Text-fig. 9):

1. Pallial sinus extending forward of the mid-point of the shell. Lower limb of pallial scar (below sinus) long and narrow. Inside white, often stained with purple posteriorly. 2
   Pallial sinus usually not reaching mid-point of shell. Lower limb of pallial scar usually wedge-shaped. 3
2. Outside with fine radial and concentric sculpture. Concentric ribs little modified posteriorly. In sand or gravel, attached to small stones by a byssus. PULLASTRA
   Concentric ribs becoming foliaceous posteriorly. Radial sculpture present, but may be worn away in some specimens. Shape irregular, rather elongated. In rock crevices. SAXATILIS
3. Radial sculpture as distinct or more distinct than concentric sculpture. Lower limb of pallial scar wedge-shaped. Outside matt. DECUSSATA
   Radial sculpture absent or restricted to faint irregular grooves. Lower limb of pallial scar variable. Outside slightly glossy. 4



Text-fig. 9. Inside of right valves of *Venerupis* shells, showing pallial and adductor scars. A, *V. pullastra*, with line showing mid-point of shell; B, *V. decussata*; C, *V. rhomboides*; D, *V. aurea*. × ½, except D which is × ¾.

4. Inside white or cream, tinged with yellow. Length up to 4 cm. Fairly thin-shelled, triangulo-ovate, somewhat inflated. Outside cream-coloured, usually with liver-coloured markings. Lunule well defined, bounded by a fine groove. Pallial sinus reaching to about mid-point of shell. Faint radial sculpture often present. AUREA
   Inside white, often tinged with pink. Length 5 cm or more. Shell thick, triangulo-ovate or rhomboidal. Outside creamy white, often with pink or reddish brown markings. Lunule sometimes poorly defined, not bounded by a clearly marked groove. Radial sculpture absent. Pallial sinus does not extend to mid-point of shell. RHOMBOIDES

Note. There has been some doubt as to the separate identity of *saxatilis*, and it may be difficult to distinguish from *pullastra* where the habitat is not known. The shape of the pallial sinus in *aurea* is variable. It is usually rather rounded, with a narrow V-shaped lower limb.

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THE BRITISH SPECIES OF *THRACIA* (EULAMELLIBRANCHIA)

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(Plate I and Text-figs. 1–5)

Inspection of fauna lists suggests that many authors have found difficulty in identifying the British species of *Thracia*, most being content to list them as *Thracia* spp. Examination of museum collections and even keys to identification (Ziegelmeier, 1957) shows many errors of identification. The problem was brought to notice when some specimens dredged off the Northumberland coast appeared to differ from the five species recognized as occurring in British waters. It was decided that a study of the specific differences similar to that of Holme (1951, 1959) for *Ensis* and *Lutraria*, and Allen (1954) for *Nucula*, would be useful.

Specimens, particularly undamaged ones, of the British species of *Thracia* are difficult to obtain because they are both fragile and rare, and I am greatly indebted to the many people who were so kind as to give material and information for this study. These included Mr J. H. Barrett, Dale Fort Field Centre; Dr C. Burdon-Jones, Marine Biology Station, Menai Bridge; Mr I. C. J. Galbraith, British Museum (Nat. Hist.); Prof. R. A. R. Gresson, Queen's University, Belfast; Prof. L. A. Harvey, The University, Exeter; Mr N. A. Holme, Marine Biological Association, Plymouth; Dr N. S. Jones, Marine Biological Station, Port Erin; Mr A. D. McIntyre, Scottish Home Department, Aberdeen; Mr R. McLaughlin, The University, Aberdeen; Dr R. H. Millar and Dr R. B. Pike, Scottish Marine Biological Association, Millport; Mr A. M. Tynan, Hancock Museum, Newcastle upon Tyne. I also wish to thank Dr D. A. Edwards, King's College, Newcastle upon Tyne, for assistance in matters mathematical.

Winckworth (1932) recognizes five British species of *Thracia*. Four of these—*T. phaseolina* Lamarck, *T. villosiuscula* Macgillivray, *T. pubescens* (Montagu), *T. convexa* (W. Wood)—are included in the subgenus *Thracia* and the other, *T. distorta*, in the subgenus *Ixartia*. There has been, and still is, considerable variation of opinion as to whether *T. phaseolina* and *T. villosiuscula* are varieties of the same species. Reference to the most used accounts of the British Mollusca shows that while Jeffreys (1862–9) considers *T. villosiuscula* as a variety of *T. papyracea* (= *T. phaseolina*), Forbes & Hanley (1848–53) treat them as a separate species. The drawing of *T. villosiuscula* in Forbes & Hanley is one of the few that are inaccurate, but fortunately Macgillivray's collection

of species of *Thracia* from which he described *T. villosiuscula* (Macgillivray, 1827) exists in Aberdeen University. Jeffreys (1862–9) also refers to a second variety, *T. phaseolina* var. *gracilis*, which he does not figure, nor is there any trace of a type specimen either in Britain or in the Jeffreys collection in the National Museum, Washington, U.S.A. It has been suggested that *T. phaseolina* is the young of *T. pubescens* (Forbes & Hanley, 1848–53). This suggestion is made mainly on the grounds of similarity in shape and that very few young specimens of *T. pubescens* have been taken. No small specimens were available for this investigation, although examination of the growth lines in the large specimens gives a good indication of the shape of the young shell. Surprisingly, many specimens of *T. convexa* are labelled *T. pubescens* in museum collections.

DISTRIBUTION

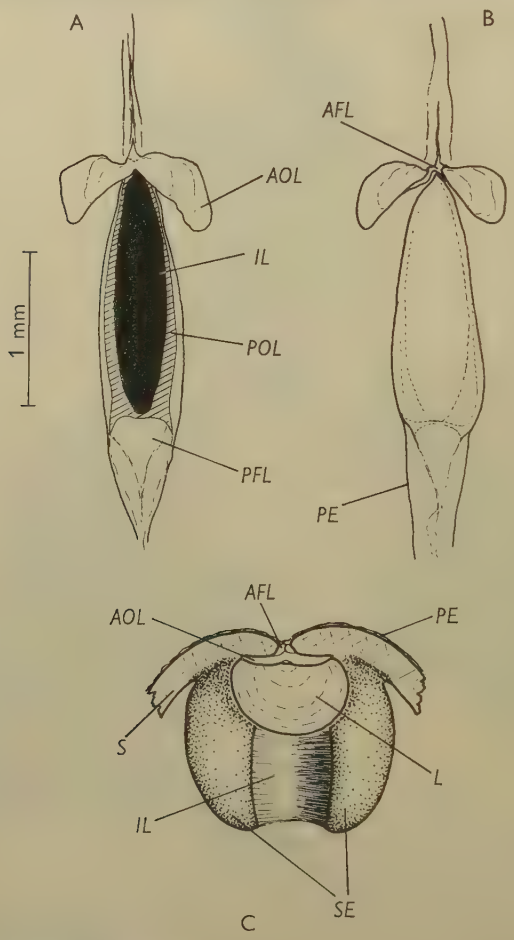
In British waters *T. pubescens* is restricted to the English Channel off the coasts of Dorset, Devon and Cornwall. Specimens of *T. pubescens* recorded from Belfast (Jeffreys, 1862–9) are almost certainly misidentifications. The remaining species are found throughout British territorial waters, with the possible exception of the area south of the Humber to Southampton water. Outside British waters in the eastern Atlantic, all but *T. pubescens* are found from the west coast of Norway south to Madeira, and also throughout the Mediterranean (Kobelt, 1886–8). *T. pubescens* extends no farther north than the south coast of Britain, but its southern limits are similar to those of the other species. Although the geographical limits of the British species are similar, their environmental requirements vary. They usually occur sublittorally, but at certain localities *T. villosiuscula*, *T. phaseolina* and, more rarely, *T. distorta* occur on the shore at ELWST. *T. distorta* is found in rock crevices, it is not a borer nor is it attached by its byssus when adult and its shell form depends largely on the limitations of the crevice. The remaining species live in softer substrates, *T. phaseolina* occurs mainly in sands and muddy sands, *T. villosiuscula* in sandy gravels, *T. pubescens* and *T. convexa* in muds and sandy muds.

THE SPECIFIC DIFFERENCES

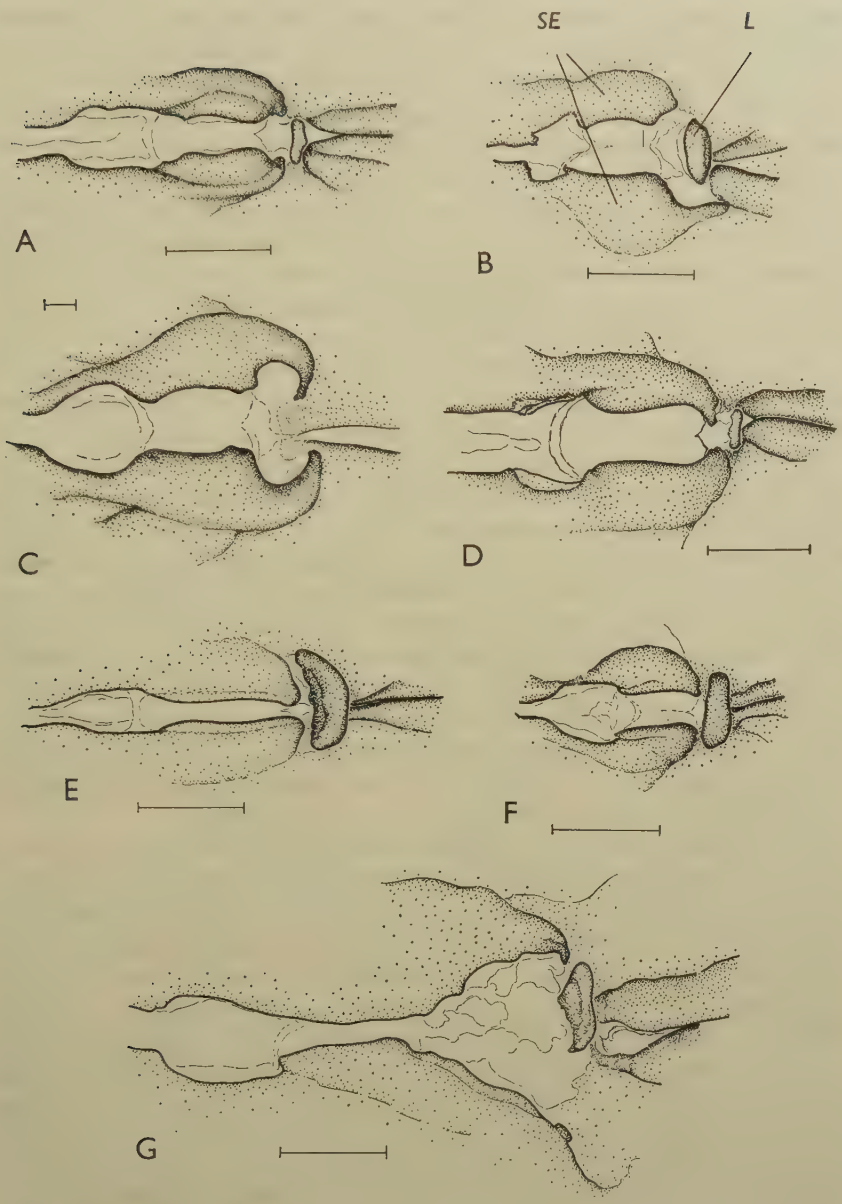
Species of the subgenus *Thracia* can only be identified with certainty by reference to the shell and ligament. Differences in the soft parts (numbers of siphonal papillae, form of the gills, foot, etc.) are too slight to be used with accuracy. However, *T.* (*Ixartia*) *distorta*, in addition to its marked differences in shell characters, differs from the other British species in the shallowness of its siphonal embayment and the shortness of its siphons. It also possesses a large number of siphonal tentacles (18–24). Unfortunately no living *T. pubescens* have been examined, but the remaining British species have between 4 and 8 tentacles to each siphon.

Text-fig. 1. Chart showing the various localities from where the specimens examined in this study were taken. It also includes the localities of past records of *Thracia phaseolina* var. *gracilis*. A, *T. villosiuscula*; B, *T. phaseolina* var. *gracilis*; C, *T. phaseolina*; D, *T. convexa*; E, *T. distorta*; F, *T. pubescens*.

All British species of *Thracia* are inequivalve with the right valve larger than and overlapping the left (Pl. I). The shell is either white or cream coloured, with a matt surface and, with the exception of *T. distorta*, they are extremely fragile without very well marked growth lines. *T. distorta* has a relatively thick shell with coarse, unevenly shaped growth lines (Pl. I). The shell surface is ornamented with microscopic pustules which tend to be denser at the posterior part of the shell and, in older specimens, those in the central part of the shell are often worn away. *T. distorta* and *T. pubescens* have relatively large, coarse pustules numbering approximately 150–200/mm^2 and 75–150/mm^2



Text-fig. 2. The ligament of *T. villosiuscula*. A, Internal view; B, external view; and C, a semi-diagrammatic view of the lithodesma and spoon-shaped extensions of the shell as seen from the anterior end of the shell, the valves being broken transversely at the umbo. *AFL*, anterior fusion layer; *AOL*, anterior outer layer; *IL*, inner layer; *L*, lithodesma; *PE*, periostracum; *PFL*, posterior fusion layer; *POL*, posterior outer layer; *S*, broken valve; *SE*, spoon-shaped extension.



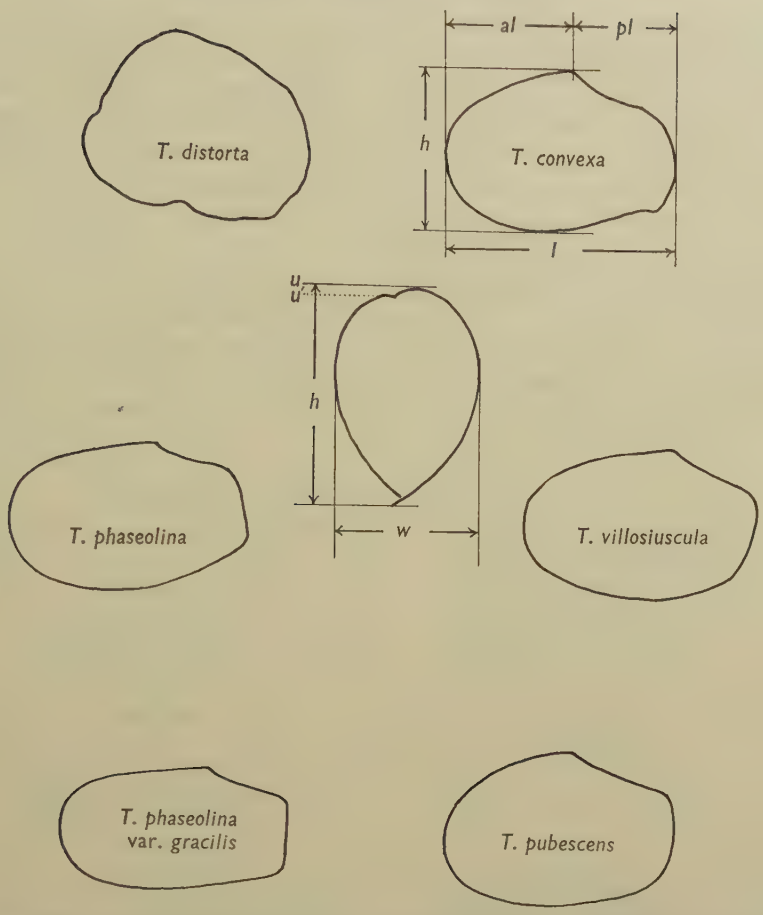
Text-fig. 3. Internal views of the hinge and ligament of A, *T. convexa* (total length of the shell 20·5 mm); B, *T. distorta* (16·5 mm); C, *T. pubescens* (85 mm), the position of the lithodesma is outlined in dots; D, *T. phaseolina* var. *gracilis* (18·5 mm); E, *T. villosiuscula* (13·2 mm); F, *T. phaseolina* (9 mm); G, *T. phaseolina* (26 mm). Lettering as in Text-fig. 2. Scales represent 1 mm.

respectively, the other three species have much finer pustules: *T. villosiuscula* with about 600–900/mm^2, *T. phaseolina* 1500–2500/mm^2 and *T. convexa* 2000–3000/mm^2. There is fairly wide intraspecific variation in density.

The hinge and ligament is essentially the same as that described for *Cochlodesma pretenue* (Allen, 1958, 1960). There are no hinge teeth, the ligament being held by a pair of internal spoon-shaped extensions of the shell (Text-fig. 2). These extensions are longer than those in *Cochlodesma* and the length of the inner and posterior outer layers of the ligament is correspondingly increased (Text-figs. 2 and 3). Immediately anterior to the spoon-shaped extension lies the roughly semicircular lithodesma which is attached to the lateral wings of the much modified and thickened anterior outer layer (Text-figs. 2 and 3). Although not discussed in previous papers, a lithodesma is also present in *Cochlodesma* (Allen, 1958, 1960) and, as in the latter genus, the anterior fusion layer is bifurcate in *Thracia* but only to a slight extent.

There are specific differences in the form of the hinge and ligament (Text-fig. 3) which mainly involve the length of the spoon-shaped extensions and the size of the lithodesma. The extensions are shortest in *T. distorta*, *T. pubescens* and *T. convexa*, with the two latter species possessing the smallest lithodesma. The spoon-shaped extensions of *T. villosiuscula* and *T. phaseolina* are longer, those of *T. villosiuscula* being approximately half as long again as in *T. phaseolina* of the same shell length. Similarly, the lithodesma of *T. villosiuscula* is twice the size of that of *T. phaseolina*. The difference in the length of the ligament of these two species is apparent externally. Forbes & Hanley (1848–53) state that the lithodesma (= ossicle) of *T. phaseolina* is broadest in the centre while that of *T. villosiuscula* is no broader in the centre than at the sides. This has not been confirmed.

The best single character on which to distinguish the British species of *Thracia* is the outline of the shell in lateral view (Pl. I, Text-fig. 4). Although the specimens examined came from widely differing localities (Text-fig. 1) the shape of the shell outline of each species was remarkably constant, even in small shells 3–4 mm long. The differences seen in Pl. I and Text-fig. 4 are self-descriptive and few comments need be made. As might be expected, shell shape is most variable in the crevice dweller *T. distorta*, but the indentation of the ventral edge appears to be more than a distortion due to cramped space for it is present to a greater or lesser degree in all specimens examined over 3–4 mm long (cf. Text-fig. 4 and Pl. I). In addition, *T. distorta* is the only species in which the pre-umbonal length is shorter than the post-umbonal length. The differences in the curvature of the posterior shell margins of *T. phaseolina* and *T. villosiuscula* are remarkably constant and no intermediates were found, although they might be expected to occur if these were but varieties of the same species. The shell outline of *T. pubescens* approaches much more closely to that of *T. villosiuscula* than it does *T. phaseolina*, but the difference in the position of the umbo is distinctive. In the case of



Text-fig. 4. The shell outline of the British species of *Thracia*. *al*, pre-umbonal length; *h*, height; *l*, total length; *pl*, post-umbonal length; *w*, width; *u*, true point of height measurement; *u'*, umbo.

T. convexa, the distinctive posterior shell margin is an infallible diagnostic character as is the great inflation of the shell valves.

An attempt has been made to determine the differences in shape quantitatively and to test the significance of the differences. Five measurements were made on each shell (Text-fig. 4) namely: total length (*l*), height (*h*), width (*w*) pre-umbonal (*al*) and post-umbonal length (*pl*). From these measurements four ratios were calculated—*w*/*l*, *h*/*l*, *h*/*w*, *al*/*pl*—for each specimen. Assuming the height measurement is taken from the umbo[1] and that

[1] In practice, because the normal axis (Owen, 1953; Allen, 1954) is a logarithmic spiral, the height is measured from above the umbo. As the spiral angle is very small the distance between the true point of measurement and the umbo is small and can be neglected (note points *u* and *u'* in Text-fig. 4).

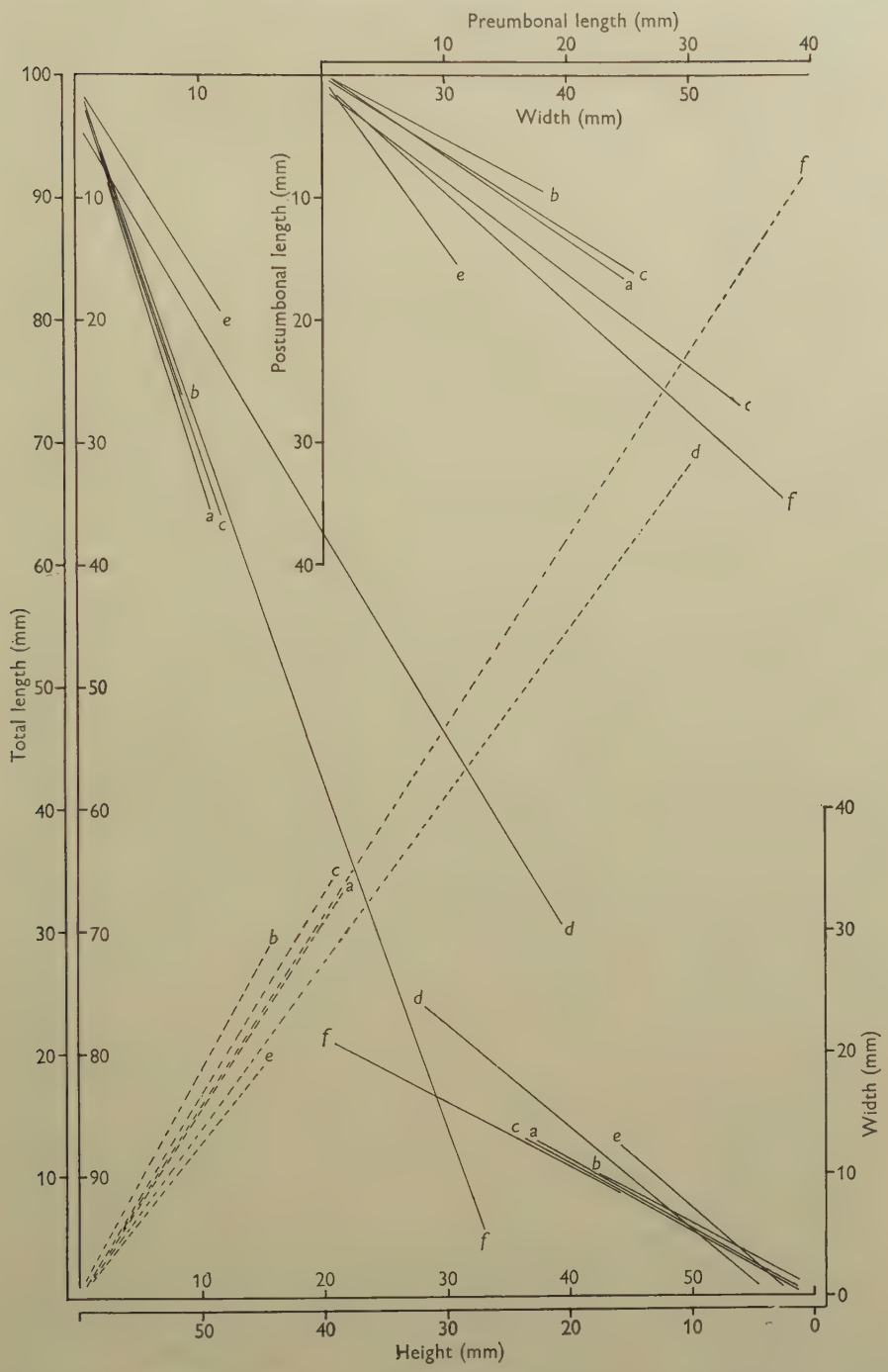
the shell increments are gnomons then the regressions of w on l, h on l, w on h, and al on pl, will be linear. This was confirmed and the various regressions are shown in Text-fig. 5 and Table 1. For clarity the individual points are omitted, the limits being shown in Table 2. From these measurements it is clear that *T. convexa* and *T. distorta* can be easily separated from the other species by the greater convexity of their shell valves. In both species the width (w) averages half the length, while in the case of *T. phaseolina*, *T. villosiuscula* and *T. pubescens* the width averages a third of the length. It is also clear that the height measurement relative to the width and the length is significantly greater in *T. convexa* and *T. distorta* than the other species (Table 2). *T. distorta* and *T. convexa* are separated easily because the pre-umbonal length of *T. distorta* is less than the post-umbonal length while the reverse is true of *T. convexa*.

TABLE 1. SAMPLE REGRESSION EQUATIONS

l, total length; w, width; h, height; al, pre-umbonal length; pl, post-umbonal length; measurements in mm, see Text-fig. 5.

T. phaseolina var. *gracilis*	*T. phaseolina*
$l = 19{\cdot}34 + 3{\cdot}12(w - 6{\cdot}16)$	$l = 11{\cdot}61 + 3{\cdot}03(w - 3{\cdot}63)$
$h = 10{\cdot}39 + 1{\cdot}86(w - 6{\cdot}16)$	$h = 7{\cdot}02 + 1{\cdot}79(w - 3{\cdot}63)$
$l = 19{\cdot}34 + 1{\cdot}84(h - 10{\cdot}39)$	$l = 11{\cdot}61 + 1{\cdot}68(h - 7{\cdot}02)$
$pl = 7{\cdot}20 + 0{\cdot}64(al - 12{\cdot}14)$	$pl = 4{\cdot}50 + 0{\cdot}66(al - 4{\cdot}50)$
T. villosiuscula	*T. convexa*
$l = 14{\cdot}93 + 2{\cdot}77(w - 5{\cdot}26)$	$l = 33{\cdot}19 + 1{\cdot}72(w - 17{\cdot}37)$
$h = 9{\cdot}65 + 1{\cdot}74(w - 5{\cdot}26)$	$h = 24{\cdot}29 + 1{\cdot}20(w - 17{\cdot}37)$
$l = 14{\cdot}93 + 1{\cdot}55(h - 9{\cdot}65)$	$l = 33{\cdot}19 + 1{\cdot}37(h - 24{\cdot}29)$
$pl = 5{\cdot}92 + 0{\cdot}68(al - 9{\cdot}01)$	$pl = 14{\cdot}77 + 0{\cdot}80(al - 18{\cdot}42)$
T. pubescens	*T. distorta*
$l = 79{\cdot}36 + 2{\cdot}91(w - 27{\cdot}36)$	$l = 11{\cdot}84 + 1{\cdot}61(w - 7{\cdot}01)$
$h = 51{\cdot}34 + 1{\cdot}88(w - 27{\cdot}36)$	$h = 9{\cdot}44 + 1{\cdot}26(w - 7{\cdot}01)$
$l = 79{\cdot}36 + 1{\cdot}55(h - 51{\cdot}34)$	$l = 11{\cdot}84 + 1{\cdot}26(h - 9{\cdot}44)$
$pl = 38{\cdot}37 + 0{\cdot}90(al - 41{\cdot}99)$	$pl = 6{\cdot}93 + 1{\cdot}40(al - 4{\cdot}91)$

Table 2 and Text-fig. 5 show that the measurements of *T. villosiuscula*, *T. phaseolina* and *T. pubescens* are very close, and tests of significance were carried out between pairs of these species for each of the ratios calculated (Table 3). Although the numbers of *T. villosiuscula* and *T. phaseolina* are significantly large (69 and 85 respectively), because of the small numbers of the other species, 'Student's t-distribution' was used. The probabilities that the various ratios differ significantly from each other are given in Table 3. When the ratio of pre-umbonal length to post-umbonal length alone is considered it can be seen that the probability that the three species differ significantly from each other is to the 99·9% limit. But for this ratio, it would seem from *measurements alone* that *T. pubescens* and *T. villosiuscula* do not differ significantly from each other. Except for the width/height ratio



Text-fig. 5. Relations between height, width and length, and pre-umbonal and post-umbonal length in the British species of *Thracia*; regression lines drawn. *a*, *T. villosiuscula*; *b*, *T. phaseolina* var. *gracilis*; *c*, *T. phaseolina*; *d*, *T. convexa*; *e*, *T. distorta*; *f*, *T. pubescens*.

TABLE 2. COMPARISON OF THE RATIOS WIDTH TO LENGTH (*w/l*), WIDTH TO HEIGHT (*w/h*), PRE-UMBONAL LENGTH TO POST-UMBONAL LENGTH (*al/pl*) AND HEIGHT TO LENGTH (*h/l*) IN THE BRITISH SPECIES OF *THRACIA*

r, Maximum and minimum values recorded; $\bar{x}$, sample mean and *s*, its standard deviation

		w/l			*w/h*			*al/pl*			*h/l*		
	Numbers	$\bar{x}$	*s*	*r*	$\bar{x}$	*s*	*r*	$\bar{x}$	*s*	*r*	$\bar{x}$	*s*	*r*
T. pubescens	9	0·347	0·021	0·32–0·38	0·535	0·021	0·51–0·57	1·12	0·07	0·96–1·29	0·650	0·030	0·62–0·69
T. phaseolina	85	0·311	0·034	0·21–0·40	0·504	0·062	0·33–0·70	1·73	0·37	1·21–2·77	0·622	0·035	0·51–0·75
T. villosiuscula	69	0·355	0·025	0·32–0·44	0·538	0·050	0·47–0·72	1·59	0·24	1·08–2·19	0·661	0·034	0·58–0·75
T. phaseolina var. *gracilis*	10	0·316	0·025	0·27–0·34	0·589	0·048	0·51–0·68	1·74	0·26	1·37–2·05	0·539	0·015	0·52–0·57
T. convexa	29	0·507	0·053	0·45–0·63	0·669	0·058	0·57–0·81	1·29	0·18	1·07–1·82	0·734	0·044	0·60–0·82
T. distorta	14	0·635	0·121	0·44–0·87	0·721	0·192	0·44–1·06	0·72	0·12	0·50–0·93	0·789	0·066	0·67–0·93

TABLE 3. PROBABILITIES (*P*) THAT VARIOUS PAIRS OF SPECIES DIFFER FROM EACH OTHER

T.v., *T. villosiuscula*; *T.ph.*, *T. phaseolina*; *T.pub.*, *T. pubescens*; *T.ph.g.*, *T. phaseolina* var. *gracilis*.

	T.ph./ T.ph.g.	*T.v./ T.ph.g.*	*T.ph./ T.pub.*	*T.v./ T.pub.*	*T.v./ T.ph.*
w/l	0·6	0·001	0·01	0·9	0·01
w/h	0·001	0·01	0·2	0·9	0·3
al/pl	0·9	0·1	0·001	0·001	0·001
h/l	0·001	0·001	0·05	0·9	0·01

the members of both other pairs, *T. villosiuscula–T. phaseolina* and *T. phaseolina–T. pubescens* differ significantly from each other (Table 3).

Thus, examination shows that the five species listed by Winckworth (1932) are valid. All that remains to be considered is the identity of the specimens dredged from off the Northumberland coast.

THRACIA PHASEOLINA VAR. *GRACILIS* JEFFREYS

Apart from the five species considered so far, the only other British *Thracia* that is recorded in conchological literature is *T. phaseolina* var. *gracilis*. Jeffreys (1862–9) describes this variety as: 'Shell more slender [than *T. phaseolina*], and approaching a cylindrical shape, thinner, more uniformly convex; posterior end shorter in proportion'. He does not figure this shell nor has a type specimen been found. Jeffreys records two localities, Bantry Bay and 20 miles north of Unst in 20 fm. (Text-fig. 1). The only other record found in the literature is one by Leckenby & Marshall (1875) from the Dogger Bank in 20 fm. Examination of the Northumberland specimens, taken in 20–22 fm. in muddy gravel, 2 miles, 050° from Newbiggin Church, Northumberland (55° 13′ N., 1° 27½′ W.), shows that they most closely resemble *T. villosiuscula* and *T. phaseolina* (Pl. I, Text-figs. 3–5 and Tables 1–3). The shells of the Northumberland specimens are pure white and extremely fragile, with a matt periostracum, pustules number about 700–1500 mm^2. The hinge and ligament are similar to *T. phaseolina* although with a smaller lithodesma (Text-fig. 3). The shell outline is quite distinct from the other British species. It can be seen that the height measurement (h/l, h/w ratios, Tables 2 and 3) is much less than the other species and that the posterior end is characteristically truncate. However, the ratio al/pl is not significantly different from *T. phaseolina* (cf. Jeffreys's description of var. *gracilis*). Measurements show that the Northumberland specimens differ significantly from *T. villosiuscula* and somewhat less significantly from *T. phaseolina*. The very straight ventral shell margin lying parallel to the dorsal margin and the truncated posterior margin give the specimens a cylindrical appearance. There seems little doubt that they correspond with the description given by Jeffreys.

Were it not for the fact that the original description was of specimens taken from localities as far apart as Bantry Bay and Unst and did not include North Sea specimens, one would perhaps incline to the view that the Northumberland specimens were a local variation of *T. phaseolina*, similar to those reported for other lamellibranchs (Allen, 1953, 1954). The eggs in *T. phaseolina* are brooded in the mantle cavity and the larva is either non-planktonic or an extremely short-lived planktonic type. Dispersion will tend to be minimal and there will be a tendency towards inbreeding, factors likely to encourage local variation. Yet, the shell form of *T. phaseolina* taken from a wide variety of habitats remains surprisingly constant. Unfortunately no

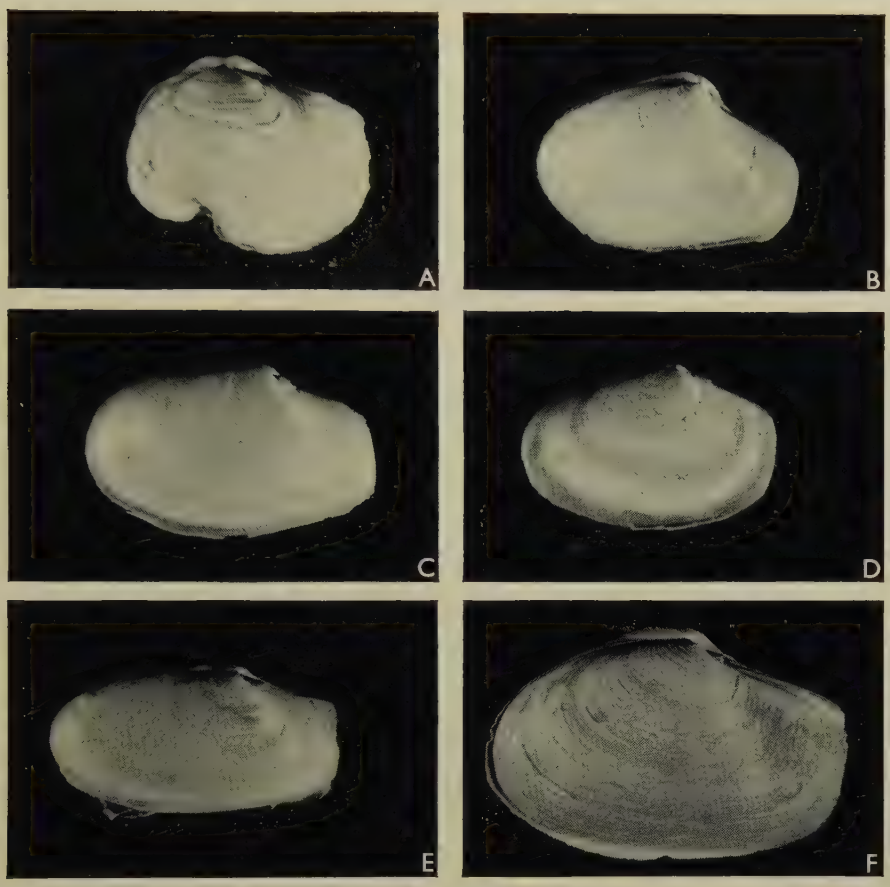
specimens of the variety were obtained from elsewhere and until more specimens are available it would be wise to defer a decision as to whether this is a true species and refer to the Northumberland specimens as *T. phaseolina* var. *gracilis*. A type specimen has been lodged in the U.S. National Museum, Washington, where the Jeffreys collection is housed.

CONCLUSIONS

The conclusions to be drawn from this study can best be made in the form of a key to the species to be used in conjunction with Pl. I, Text-figs. 3 and 4, and Table 2.

KEY

1. Pre-umbonal length greater than post-umbonal length. 2
   Post-umbonal length greater than pre-umbonal length. Shell not very fragile, often distorted; coarse, irregular, growth lines; ornamented with relatively coarse pustules, approx. 150–200/mm^2; ventral shell margin indented to a greater or lesser extent anteriorly in shells longer than 3 mm; width of shell more than half the total length; height/length ratio greater than other British species of *Thracia*. Ligament very short. Siphons relatively short; siphonal embayment shallow; 18–24 tentacles. Found in sublittoral rock crevices. Maximum recorded length 18 mm. *T. distorta*
2. Shell not greatly inflated, width approx. 1/3 of length. 3
   Shell greatly inflated in the central part, width 1/2 that of length. Shell very fragile, white in young specimens (3 cm), cream in older specimens; ornamented with very fine pustules, approx. 2000–3000/mm^2; umbos prominent, posterior dorsal shell margin sloping steeply from the umbos, posterior ventral shell margin may have slight indentation. Ligament short, lithodesma small. Always sublittoral, in muds and sandy muds. Maximum recorded length 67 mm. *T. convexa*
3. Shell white, ornamented with very fine pustules. 4
   Shell cream or sand coloured; ornamented with relatively coarse pustules, approx. 75–150/mm^2. Pre-umbonal length only slightly longer than the post-umbonal length (*al*/*pl*, av. 1·12). Ligament relatively short, lithodesma small. Always sublittoral, in muds and sandy muds off the south coasts of Dorset, Devon and Cornwall. Maximum recorded length 96 mm. *T. pubescens*
4. Height measurement relatively small (height/length ratio, av. 0·54) ventral shell margin almost straight, posterior margin sharply truncate and at right angles to the long axis of the shell. Ligament relatively long, lithodesma small. Shell fragile, white; ornamented with pustules, approx. 700–1500/mm^2. Sublittoral, from muddy gravels. Maximum recorded length 27 mm. *T. phaseolina* var. *gracilis*
   Ventral shell margin rounded, posterior shell margin truncate but at an angle to the long axis, the posterior corner of the ventral shell margin in advance of the dorsal. Ligament relatively long, lithodesma fairly large. Shell fragile, white; ornamented with pustules, approx. 1500–2500/mm^2. Sublittoral, but occasionally taken at ELWST, in sand and muddy sand. Maximum recorded length 32 mm. *T. phaseolina*



The British species of *Thracia*, photographed from the left side. A, *T. distorta*; B, *T. convexa*; C, *T. phaseolina*; D, *T. villosiuscula*; E, *T. phaseolina* var. *gracilis*, and F, *T. pubescens*.

(*Facing p.* 734)

Posterior shell margin rounded, and in typical specimens the dorsal posterior corner of the shell margin is in advance of the ventral. Relatively long ligament, length for length the longest of the British species of *Thracia*, lithodesma large. Shell fairly fragile, white, but occasional specimens with cream or sand-coloured band in the same plane as the lines of growth; ornamented with pustules, approx. 600–900/mm^2. Sublittoral, but occasionally taken at ELWST in coarse sand or gravel. Maximum length recorded 29 mm. *T. villosiuscula*

SUMMARY

Examination of specimens of the British species of *Thracia* confirms that *T. distorta*, *T. villosiuscula*, *T. phaseolina*, *T. pubescens* and *T. convexa* are distinct species. Specimens taken sublittorally off the Northumberland coast are identified with *T. phaseolina* var. *gracilis* Jeffreys, a distinct variety. The species are compared and a key given.

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AGE, GROWTH, AND REPRODUCTION OF THE ROCK GOBY, *GOBIUS PAGANELLUS* L., IN THE ISLE OF MAN

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(Plate I and Text-figs. 1–11)

Gobius paganellus is a marine gobiid (Teleostei-Percomorphi) found in the Mediterranean and along the Atlantic shores of continental Europe as far north as the Pas de Calais (Ninni, 1938; Vivien, 1939*b*). The southern limit of its range in the Atlantic appears to be at Madeira (Maul, 1949); the occurrence of this goby in the Black Sea (Steindachner, 1868) has been doubted (Slastenenko, 1939). On the British coast, the species does not extend north of the Firth of Forth or the Firth of Clyde (Holt & Byrne, 1903). In Manx waters, *G. paganellus* is common in the intertidal pools of rocky shores and may also occur among sublittoral rocks.

Although a number of exotic species have been investigated, studies on the biology of European gobies have been few, and restricted to species whose life histories differ from that of *G. paganellus*. The present work forms part of a general survey of the biology of Manx gobies, among which this fish is the only common representative of the genus *Gobius* as defined by De Buen, 1930.

MATERIAL AND METHODS

From March 1959 to September 1960, samples of *G. paganellus* were collected at Poyllbrein, a locality on the eastern side of Castletown Bay in the south of the Isle of Man. The area of shore collected over is about half a mile in length, and up to a quarter of a mile between tidemarks. The gently sloping bedrock of carboniferous limestone is crossed obliquely by numerous low ridges, and bears many intertidal pools. Poyllbrein lies in a sheltered position within the bay, and there is a rich growth of intertidal fucoids, including a wide zone of *Ascophyllum nodosum* (L.) Le Jol. Although the small Silverburn river empties into Castletown Bay, there is no faunistic indication of reduced salinity at Poyllbrein, while drainage of fresh water on to the shore is negligible. At high-water mark, there are a number of large pools which become polluted by masses of decomposing seaweed, but there are no obvious traces of this pollution in the lower pools after recession of the tide.

Collections were made at the time of low water from pools at mid-tide level and below. Post-larvae, and larger fishes from the smallest pools, were obtained by handnetting, but most specimens were secured by poisoning the

pools with Murphy Liquid Derris, a proprietary insecticide containing rotenone in emulsion. Pools chosen were of moderate size, with cover for fishes in the shape of stones and weeds, and without rapid flow of water through the pool basin. The latter feature prevented undue spread of poison into lower pools which could not have been examined in the available collecting time. Selected pools were first cleared of larger weeds and loose stones. To poison, only a small quantity of the rotenone emulsion was needed, i.e. about 10–20 ml. per m^2 of pool surface in depths of less than 30 cm. Once the poison had been dispersed by agitation of the water, and provided an excessive amount had not been added, there was relatively little cloudiness, and moribund fishes could be picked out after about 5–10 min. A thorough search of the pools was necessary to ensure collection of all the fishes, especially large *G. paganellus*, which often died in crevices or beneath stones. Occasionally individuals were found to have been partially eaten by crabs, although these and other invertebrates were themselves affected by the poison. Several pools were poisoned each month, the number depending on pool size and success in collecting. Because of the extensive intertidal area, and the abundance of *G. paganellus*, it is not believed that any serious reduction has occurred in the populations of this goby and other shore fishes as a result of poisoning.

On capture, specimens were placed immediately in 70% alcohol, and kept in this preservative until examination. Body lengths were measured to the nearest half millimetre, and exclude the caudal fin. Following removal of the gut, gonads were examined under a low-power binocular microscope, and ovaries subsequently teased up for viewing by monocular with a micrometer grid in the eyepiece for oocyte measurement. Otoliths were removed by longitudinal incision of the head and stored in 70% alcohol. After washing in absolute alcohol, and transferring to creosote, the otoliths were examined against a matt black background at low power magnification.

Since collections were usually made at the end of one month or the beginning of the next, these have been grouped about the commencement of each calendar month. Each period covers the second half of one month and the first half of the next. In some instances, material was obtained only at the end of the first month; in the graphs, results from these collections have been plotted at the beginning of the next month. The exact limits of each collecting period are given below, together with the designation used in the tables and figures:

Period 1959	Referred to as	Period 1960	Referred to as
17 Mar.–5 Apr.	Mar./Apr.	20 Jan.–8 Feb.	Jan./Feb.
15 Apr.–30 Apr.	Late Apr.	23 Feb.–10 Mar.	Feb./Mar.
19 May–3 June	May/June	21 Mar.–8 Apr.	Mar./Apr.
17 June–26 June	Late June	21 Apr.–22 Apr.	Late Apr.
16 July–7 Aug.	July/Aug.	31 May–2 June	May/June
20 Aug.–1 Sept.	Aug./Sept.	28 June–5 July	June/July
22 Sept.–23 Sept.	Late Sept.	18 July–1 Aug.	July/Aug.
23 Oct.–28 Oct.	Late Oct.	31 Aug.–1 Sept.	Aug./Sept.
23 Nov.–11 Dec.	Nov./Dec.		
21 Dec.	Late Dec.		

The seasons and quarters referred to in the text are defined as follows:

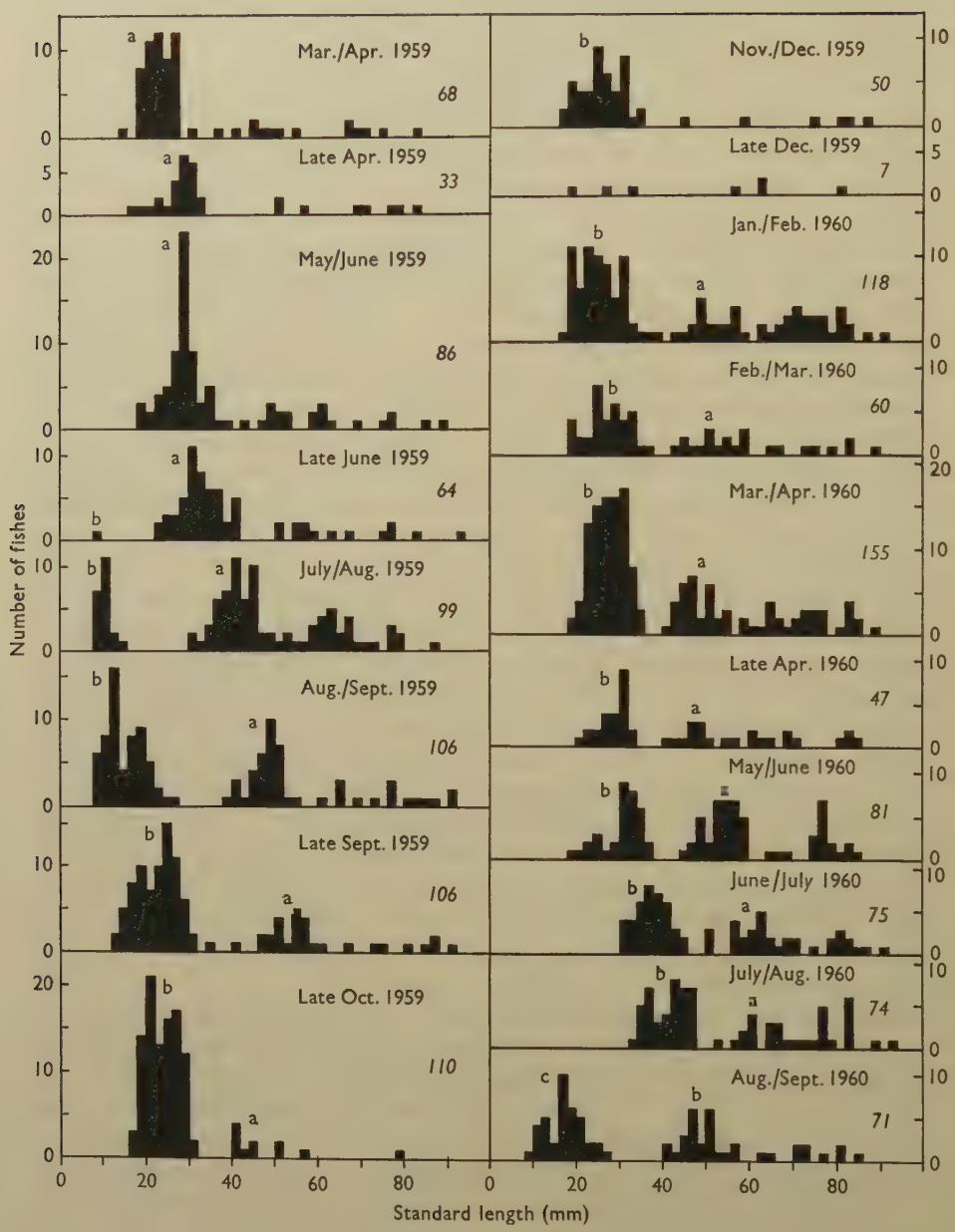
Spring	Mid-Mar.–mid-June
Summer	Mid-June–mid-Sept.
Autumn	Mid-Sept.–mid-Dec.
Winter	Mid-Dec.–mid-Mar.

AGE

From length-frequency distributions (Text-fig. 1), it was usually possible to distinguish the two youngest age-groups, and modes representing the year-classes of 1958, 1959 and 1960 could be traced through the samples. Earlier year-classes cannot be distinguished with certainty by this method, and, for more precise age-determination, annual variation in structure of the sagittal otoliths was studied. Although *G. paganellus* has scales, these were found to be unsuitable for use in estimating age. For age-determination among other gobies, the otoliths of *Pomatoschistus minutus* (Pallas) were used by Shann (1910), while the scales of *P. minutus* and a number of Japanese species have been utilized for the same purpose (Swedmark, 1958; Miyazaki, 1940; Nakamura, 1944, quoted by Takagi, 1953).

The sagittae of *G. paganellus* (Pl. I) are of the typical specialized gobioid type described by Frost (1929). They are relatively large, flattened, approximately rhomboidal bodies, lying in the vertical plane obliquely to the long axis of the brain. Otoliths have not been found in newly hatched individuals, but are present at metamorphosis of the post-larva, in which the sagittae are tiny, almost circular, and plano-convex. In small fishes, the centre or core of the otolith is usually translucent; in older examples, this may be obscured by thickening of the otolith. Growth is by deposition of concentric layers of material, producing an opaque white body in which may occur conspicuous translucent rings formed once a year in the spring. By their translucency, continuity, and relatively greater width, these *annual* rings are easily distinguished from thin translucent lines or *growth* rings between successive shells of opaque substance.

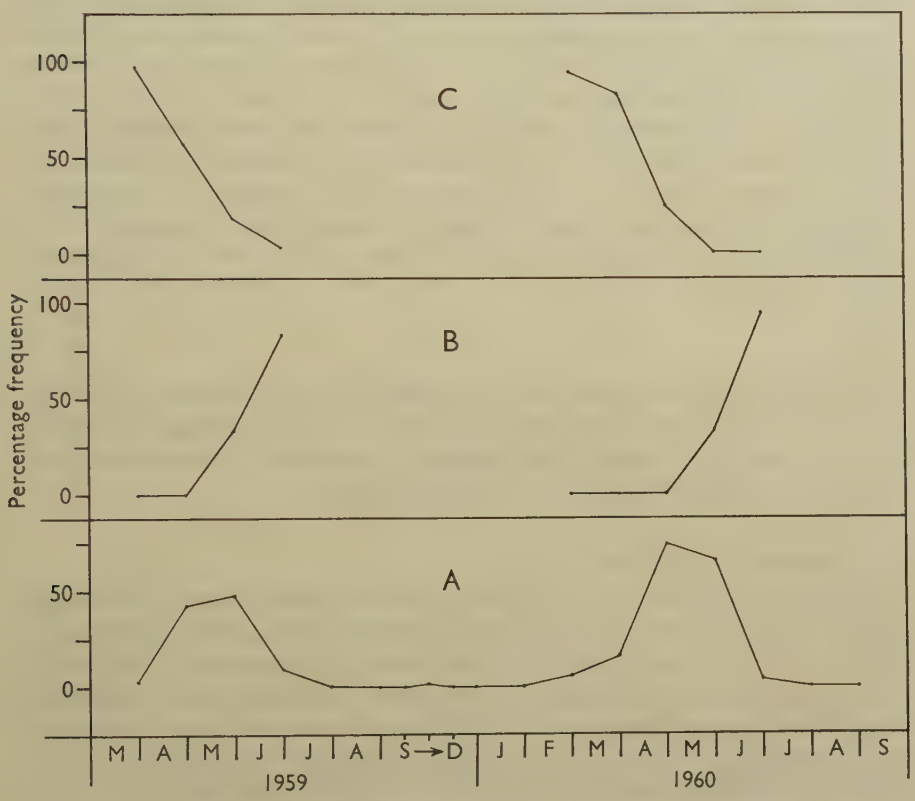
By recording the condition of the otolith edge throughout the year, it has been demonstrated that the prominent translucent rings described above are of annual formation, and age has been assessed by counting these rings. Table 1 and Text-fig. 2A show that possession of a translucent edge to the otolith is almost entirely restricted to the spring quarter, reaching maximum frequency during May. Within this period and early summer, it is possible to divide the otoliths with opaque edges into those where the latter is part of a wide peripheral opaque band (Pl. I, figs. 4, 5) or where there is no translucent ring (Pl. I, fig. 1), and those with only a very narrow edge of opaque material outside a translucent ring (Pl. 1, figs. 2, 3 and 6). Table 2 and Text-fig. 2B and C show that the latter type almost completely replaces the former



Text-fig. 1. Length–frequency histograms for each collecting period. Lengths in 2 mm size groups. Total number of fishes in each period given in italics: *a*, 1958 year-class; *b*, 1959 year-class; *c*, 1960 year-class, which was not sampled earlier in the summer.

in the course of the spring. This suggests that formation of the annual ring is probably of universal occurrence in the population.

In practice, the otoliths in this species are normally read with ease. Difficulty may be encountered in older fishes where the later annual rings tend to be crowded near the edge. Growth rings are sometimes quite conspicuous; in older specimens, they are easily recognized on comparison with the annual rings present in the same otolith, while in young fishes, lacking annual rings,



Text-fig. 2. Monthly percentage frequency of otoliths with (A) translucent edges, (B) narrow opaque periphery outside translucent ring, (C) wide opaque edge or no translucent rings.

such growth rings may be identified by their narrowness and reduced translucency. No completely illegible otolith was found in this work, and it was always possible to arrive at a fair estimate of age with difficult otoliths. Minor structural variations were noticed, such as localized excrescences on the otolith edge, especially among small fishes, but the only pronounced abnormality was the presence in an o-group fish of one otolith with an opaque core surrounded by a broad crystalline periphery.

The factors responsible for creating the annual ring in teleostean otoliths have been the subject of much speculation. In *G. paganellus*, formation of the translucent ring coincides with the spawning period and follows the period of lowest sea temperatures. For *Blennius pholis* L., another intertidal species, Qasim (1957) has shown that the annual ring is laid down during the period of gonad maturation and breeding, and after that of minimal sea temperature and food intake. In the hake, *Merluccius merluccius* (L.), Hickling (1931) found that translucency of the otolith is due to reduction in the amount of organic material incorporated into the otolith. This may be expected to happen on depletion of food reserves in the body before and during the breeding season, and could account for ring formation at this time in the adult *G. paganellus*. To explain ring formation in the immature hake, Hickling (1933) postulated the existence of an innate physiological rhythm in harmony with annual spawning exhaustion of the adults. With immature *G. paganellus*, low temperatures and perhaps a decrease in feeding may be responsible for producing the annual rings, and may also influence this process in the breeding individuals.

Longevity

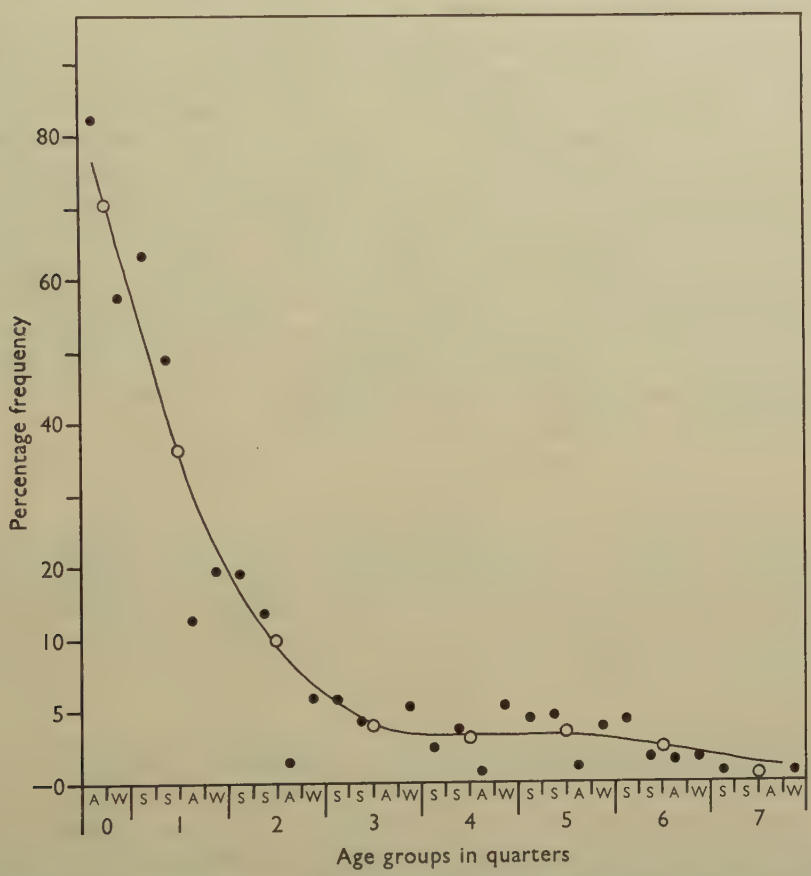
The oldest specimen taken was a 10-year-old female entering upon her eleventh summer of life. The oldest male found was 8 years of age. However, while the potential life span is thus at least 10 years, only a minute fraction of the shore population approach such an age (Table 3).

Age composition of population

Age-groups have been regarded as commencing at the start of the spring quarter to enable more natural quarterly division of the age-groups. They are referred to by the number of completed annual rings present in the otolith by the middle of the second quarter (summer) of the age-group. Immature fishes of the first two age-groups (0 and 1) were by far the largest component of the population. During spring, when breeding occurs, the older age-groups (2 and above) made up only slightly more than one-third (36·5 %) of the total numbers (Table 3). A proportion of the 2-group fishes probably did not spawn, and the certain spawners (in age-group 3 and above) comprised less than one-fifth (17·5 %) of the population. By autumn, the bulk of this was formed of young fishes (0-group) from that year's spawning, and there was possibly a sublittoral migration of older fishes which became scarce on the shore. Holt & Byrne (1898) give a sublittoral record of *G. paganellus* in Plymouth Sound, without a date. At Poyllbrein, older specimens were more numerous by the end of winter, and largely maintained their numbers in the subsequent breeding season. This increase was probably brought about by a return to the intertidal region for purposes of reproduction.

Mortality

From the relative frequency of age-groups in the population of *G. paganellus*, it was possible to derive the survival curve shown in Text-fig. 3. Dice (1952) has summarized the conditions for validity of this method. These are that (*a*) reproductive rates were constant over the years involved, (*b*) no differential mortality has occurred between the age-groups, (*c*) migration to or



Text-fig. 3. Percentage frequency of age-groups per quarter. Open circles denote average percentage frequency of each age-group. (Note that the vertical scale is expanded between 0 and 10 %.)

from the area involved has affected the age-groups to the same extent, and (*d*) sampling methods were adequate. In the present work, poisoning reduced selectivity in collecting, and the only age-group inadequately represented in the area studied would appear to be that containing the very youngest fishes, mostly still planktonic and of a size that could be easily overlooked. The

summer percentage from the 0-group has therefore been omitted from Text-fig. 3. Differences in recruitment or mortality between the respective year-classes were impossible to determine; sampling in two years of each age-group (except 0) during the spring and summer quarters may tend to reduce disparities of this nature. Older fishes tended to leave the shore by the autumn, but there was a return of the same age-groups during the following winter, and there is nothing to suggest that a majority of older fishes live permanently in the sublittoral zone.

The empirical survival curve in Text-fig. 3 indicates a high mortality rate during the first two years of life. This declines after the age of potential sexual maturity. The results for *G. paganellus* are similar to those obtained by Eschmeyer (1939) from the poisoning of an entire population of Large-Mouthed Bass, *Micropterus salmoides* (Lacepede), and the curve resembles that figured by Lack (1954) as characteristic of species living under natural conditions.

Beverton & Holt (1957) believe that predation is the most general cause of natural mortality in fish populations. Direct evidence has been obtained that *G. paganellus* is eaten by other shore fishes (*Acanthocottus bubalis* (Euphr.) and *Gaidropsarus mediterraneus* (L.)) and that small examples of this species are occasionally taken by larger *G. paganellus*. Although endoparasites were of frequent occurrence, there was no indication of pronounced mortality from the effects of parasitization or other disease.

GROWTH

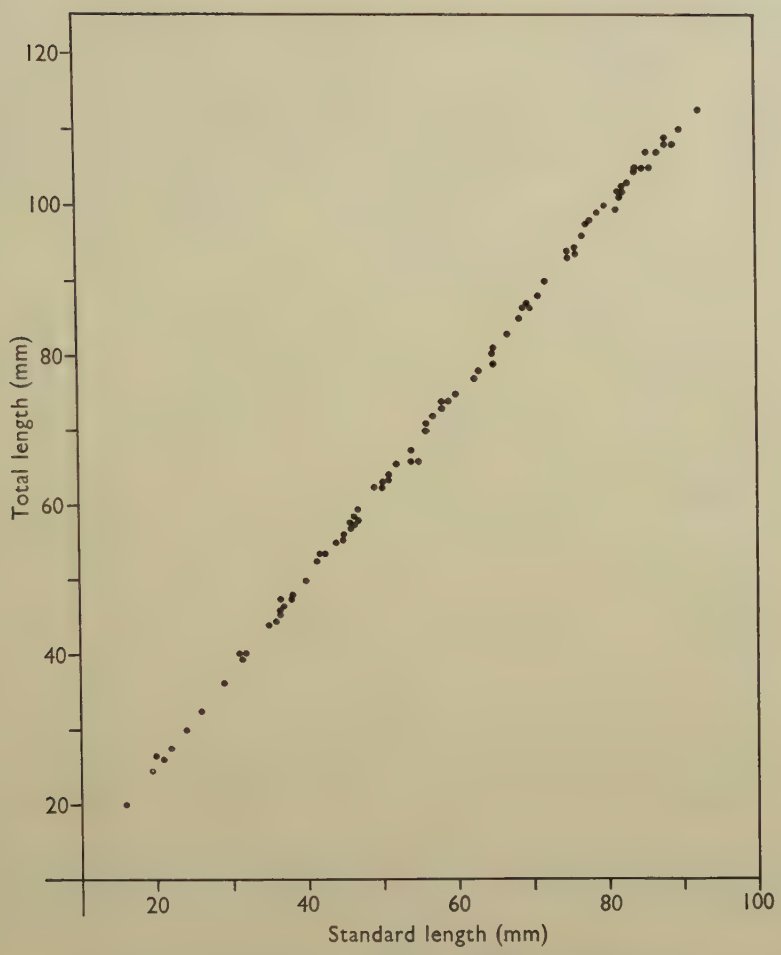
Growth is expressed here as increase in standard length, i.e. length of fish from the tip of the snout to the origin of the caudal fin. Standard length bears the isometric relation to total length (including caudal fin) shown in Text-fig. 4; in males, total length = 1·24 × standard length, in females 1·25 × standard length.

Maximum length

The largest individual obtained was a female, 92·5 mm (total length 112·5 mm); the largest male was 90·0 mm (total length 110·0 mm). These fishes belonged to age-groups 6 and 5 respectively. According to Holt & Byrne (1903), this species reaches a total length of 120 mm, which would indicate a maximum standard length of around 96·0 mm.

Growth pattern

The mean standard length of each age-group per quarter is provided for each sex in Table 4, and the average mean length in that quarter for the age-group is shown as a scatter diagram in Text-fig. 5. From the table, it can be seen that there is no consistent sexual difference in size. Growth follows a typical logarithmic pattern, with an initially high rate of growth diminishing markedly from about the age of sexual maturity (2–3 years).

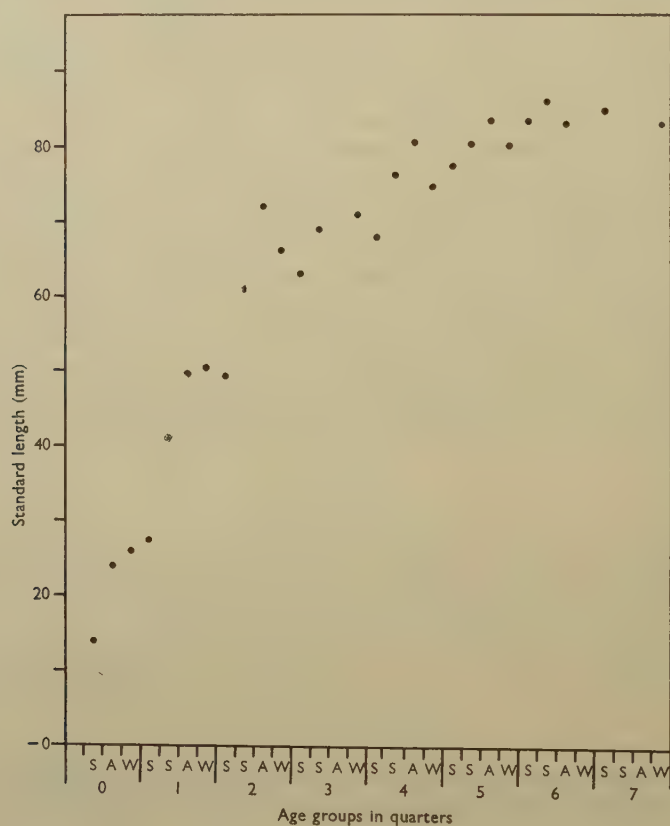


Text-fig. 4. Relation between standard length and total length.

Growth cycles

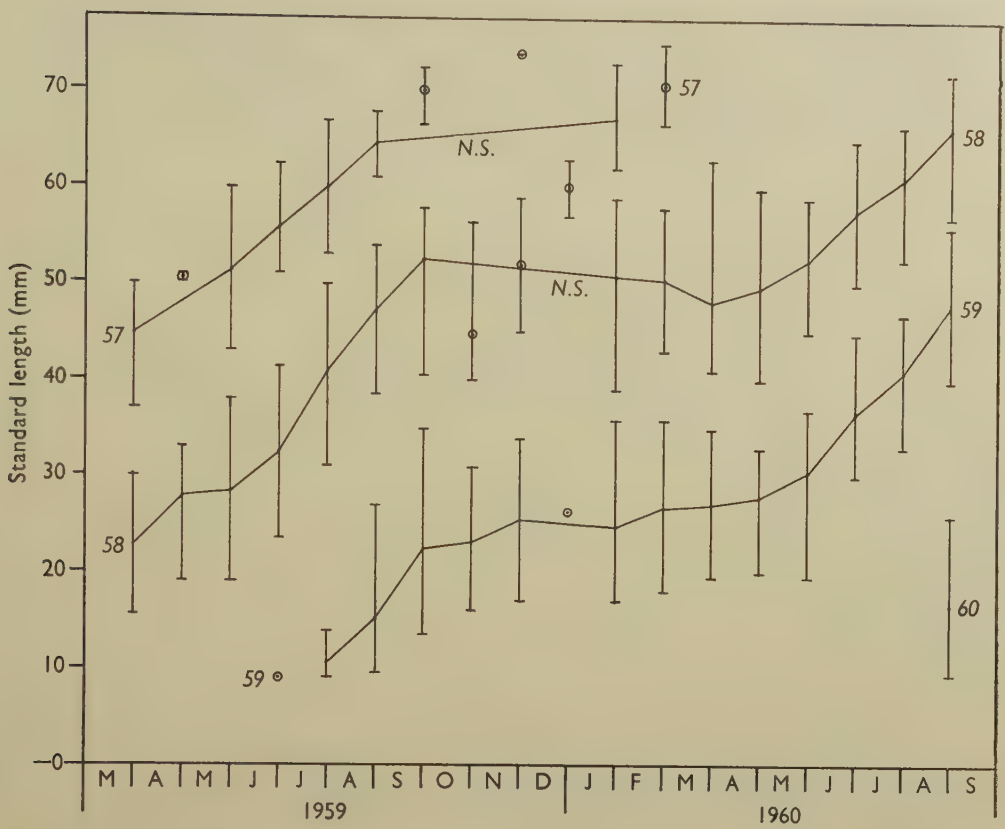
In common with other northern and temperate zone fishes, *G. paganellus* exhibits an annual cycle in growth rate. This has been studied in the first three age-groups, where numbers are usually sufficient to permit derivation of monthly mean lengths. Such values for year-classes 1957, 1958, 1959 and 1960 are given in Table 5; in plotting the average means (Text-fig. 6), those based on small samples have not been included in the growth lines. Inspection of this figure reveals that growth occurs chiefly from June to October, and to a lesser extent in April and May. The rest of the year sees little increase in length. On the whole, there is little difference in growth

between the 1958 and 1959 year-classes in age-group 1. For those of 1957 and 1958 in age-group 2, there is the possibility of better growth in the latter year-class. A growing period from spring to autumn is also indicated by quarterly means (Table 4 and Text-fig. 7) in the older age-groups. Throughout the latter, there is a more or less pronounced drop in mean length after the autumn quarter. This feature may be due to poor sampling, but the



Text-fig. 5. Quarterly mean lengths of age-groups 0–7.

regular occurrence suggests a real disappearance of the larger members of each age-group. Among the causative factors could be selective mortality of larger fishes, or a preference by these for a sublittoral environment. As shown by the slope of each growth line in Text-fig. 7, there is a typical decrease with age in the growth rate during the period of maximum growth. This change is most marked between age-groups 2 and 3. In the latter, nearly all individuals are sexually mature, while in group 2 only a proportion of the fishes reach this state (see below).



Text-fig. 6. Average monthly mean lengths for year-classes 1957–60, from March 1959 to September 1960. Ringed points denote means of samples containing fewer than 11 fishes in age-groups 0 and 1, fewer than 5 in age-group 2. Vertical lines indicate length range about each mean. N.S., no sample.

REPRODUCTION

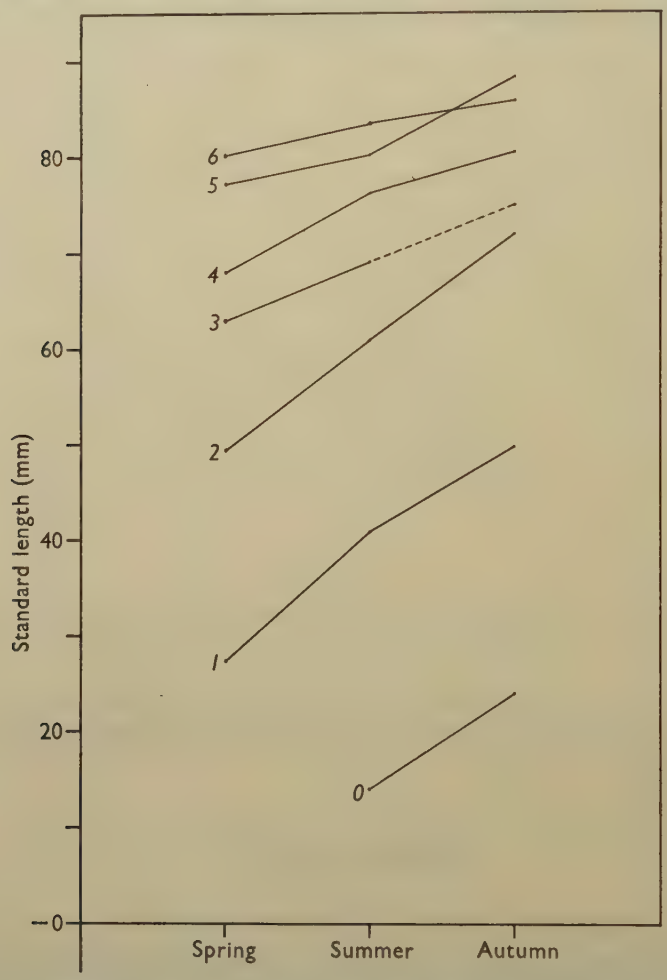
Sex ratio

In the area sampled, the overall sex ratio of females to males is 1:1·326. This preponderance of males is maintained throughout the age-groups, but the disparity in numbers between the sexes appears to be reduced in the older fishes (Table 6).

Reproductive organs

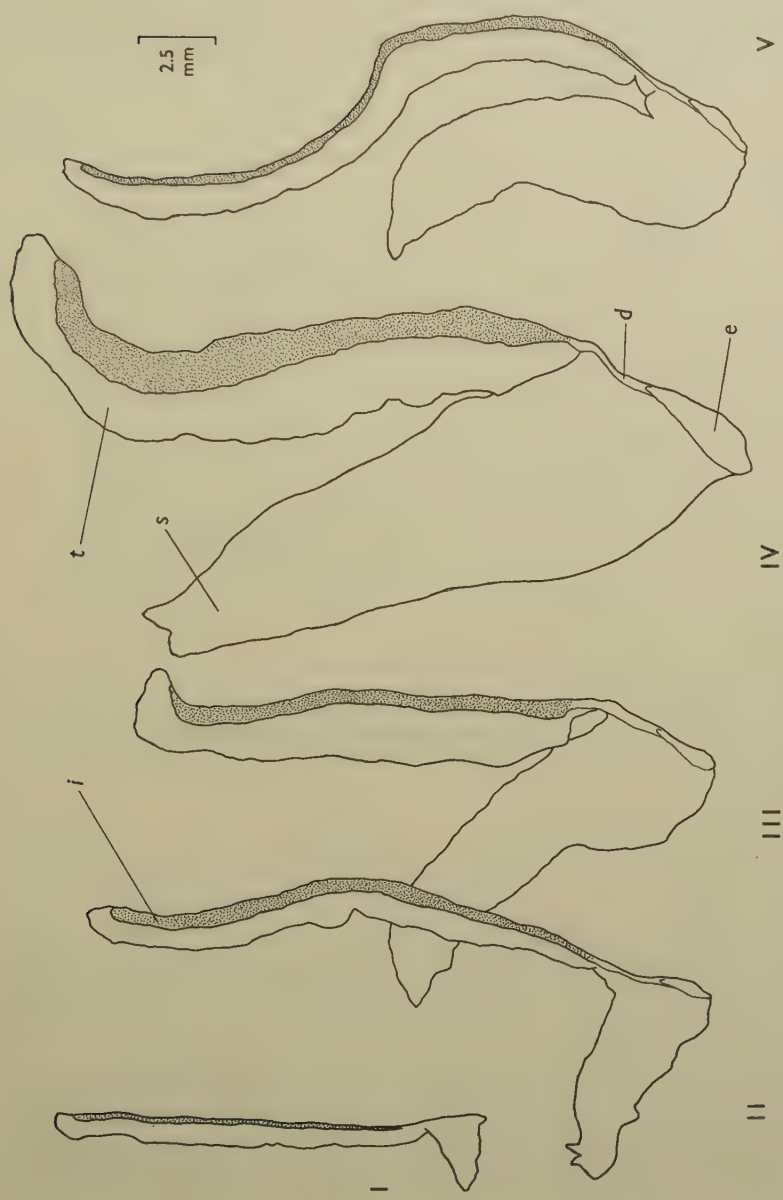
The reproductive organs of the Gobiidae have been reviewed by Eggert (1931) and Rauther (1954). The male genitalia of these fishes are specialized in the possession of large accessory glands or 'seminal vesicles' (Text-fig. 8, *s*),

opening into the sperm ducts behind the testes. The testes and seminal vesicles of *G. paganellus* have been treated in detail by Vivien (1938, 1939*b*). Vivien (1939*b*) suggested that, after ejaculation of sperm and vesicular secretion at spawning, the secretion functions to ensure contact of the sperm with the eggs and to retard dispersion of sperm into the surrounding water.



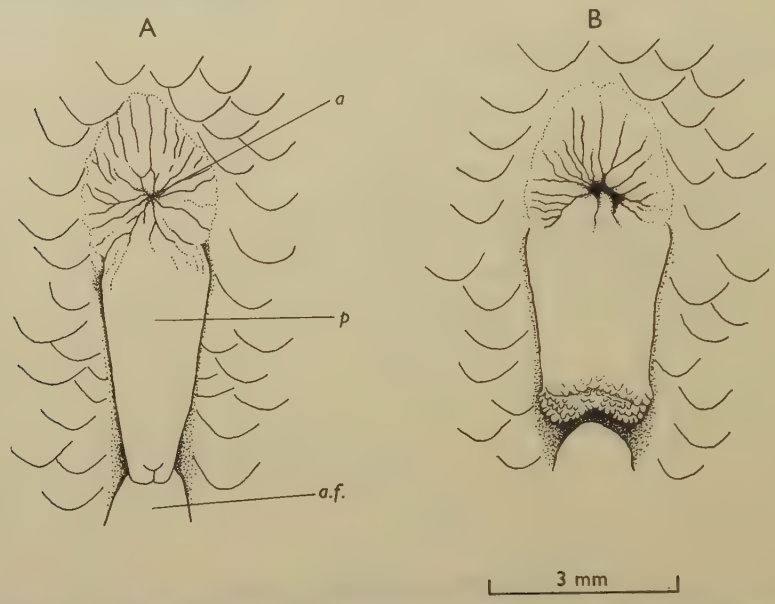
Text-fig. 7. Quarterly mean lengths over growing period for the first seven age-groups (0–6). Autumn mean for age-group 3 determined by interpolation in graph of quarterly means against age.

There is no evidence for storage of sperm in the seminal vesicles, which were found to remain entirely colourless prior to and during the breeding season (personal observation). Another peculiarity of the male reproductive system is the presence of a band of vascularized 'interstitial tissue' (Text-fig. 8, *i*),



Text-fig. 8. Maturity stages (I to V) in male genitalia. Median view of selected examples. *t*, testis lobules; *i*, interstitial tissue; *s*, seminal vesicle; *d*, sperm duct; *e*, bisected ejaculatory duct.

of unknown function, along the median side of each elongate testis. The ovary in this species, as in *Gobius niger* L. (Brock, 1878), has a central lumen, and the walls are thrown into numerous transverse folds or ovigerous lamellae. The sexes may be distinguished externally by the shape of the well developed urogenital papilla (Text-fig. 9).



Text-fig. 9. Urogenital papilla (ventral view) in mature (A) male, (B) female. *a*, anus; *p*, papilla; *a.f.*, origin of anal fin.

Development classification

The gonads were classified into arbitrary stages of maturation as follows:

Males (Text-fig. 8)

I. *Immature*. Testes usually threadlike; width less than 1·0 mm; length $\frac{1}{2}$–$\frac{2}{3}$ that of abdominal cavity (a.c.); lobules colourless and transparent. Seminal vesicles small triangular lappets; lobules inconspicuous under low-power binocular; length to apex of gland up to $\frac{1}{5}$ that of testis.

II. *Maturing virgin* and *recovering spent*. Testes narrow bands; width 1·0–1·5 mm; length $\frac{3}{5}$–$\frac{3}{4}$ a.c.; lobules faintly cloudy to dull white. Seminal vesicles larger, more or less procurved, up to half as long as testes; lobules visible.

III. *Ripening*. Testes broader bands; width 1·5–2·0 mm; length $\frac{2}{3}$–$\frac{3}{4}$ a.c.; lobules white. Seminal vesicles $\frac{1}{2}$ to nearly equal testis length, narrowing anteriorly in later stage, when ripening genitalia are difficult to distinguish from fully ripe.

IV. *Ripe*. Testes relatively wide; width 2·0–3·0 mm; length $\frac{2}{3}$ to more than $\frac{4}{5}$ a.c.; lobules opaque white. Seminal vesicles more than $\frac{2}{3}$ to equal testis length, about $\frac{1}{2}$–$\frac{2}{3}$ a.c., and broad anteriorly, becoming narrower towards end of breeding season.

V. *Spent.* Testes thin; width from more than 2·0 mm down to 0·6 mm; length from $\frac{4}{5}$ down to $\frac{3}{5}$ a.c.; lobules pale white or reddish with vascularization. Seminal vesicles typically long and narrow, less markedly vascularized than testes; length from more than $\frac{1}{2}$ down to $\frac{1}{5}$ testis length, more than $\frac{2}{3}$ down to $\frac{1}{6}$ a.c.

Females

I. *Immature.* Ovaries thin divergent bands, transparent and colourless; width up to $\frac{1}{6}$ length; length $\frac{1}{2}$–$\frac{2}{3}$ a.c. Oocytes not more than 0·125 mm in diameter.

II. *Developing virgin* and *recovering spent.* Ovaries cylindrical, divergent, translucent olive to pale yellow; width about $\frac{1}{5}$ length; length $\frac{3}{5}$–$\frac{2}{3}$ a.c. Developing oocytes faint cloudy to pale yellow; diameter 0·125–0·4 mm.

III. *Ripening.* (*a*) *Early.* Ovaries moderately swollen, in contact posteriorly, pale yellow to yellow tinged with gold; width about $\frac{1}{5}$–$\frac{1}{4}$ length; length $\frac{3}{5}$ to more than $\frac{2}{3}$ a.c. Ripening oocytes coloured as ovary; diameter 0·4–0·7 mm.

(*b*) *Late.* Ovaries swollen, in contact most of length, golden yellow; width $\frac{1}{4}$–$\frac{1}{3}$ length; length $\frac{2}{3}$ to nearly all a.c. Ripening oocytes coloured as ovary; diameter 0·7–0·95 mm.

IV. *Ripe.* Ovaries swollen, pressed together, translucent orange yellow; length nearly all a.c. Ripe oocytes transparent; diameter 0·95–1·10 mm.

V. *Spent.* Ovaries more or less flattened, yellow to translucent olive with yellow and brown bodies of various sizes; width $\frac{1}{5}$ to $\frac{1}{8}$ length; length from $\frac{2}{3}$ down to $\frac{1}{2}$ a.c.; ovary wall thickened. Ovaries contain resorbing oocytes of about 0·4 mm diameter, products of resorption, and immature or early developing oocytes of up to 0·125 mm diameter.

It is difficult to distinguish late ripening from ripe testes in alcohol-preserved material. Stage IV (ripe) in the male covers a wider range and period of sexual activity than in the female, where the ripe condition is of short duration and much more clearly defined from the preceding ripening stage.

Seasonal occurrence of developmental stages, and the breeding period of Gobius paganellus

The number of individuals at the different stages of maturity in each collecting period is shown in Tables 7–10. Ripening fishes of both sexes occurred from the end of autumn. Ripe males were found from late January to the beginning of July. Late ripening females were present by February but were not taken after June. Ripe females were obtained only in April. Spent examples of females and males appeared at the end of May and the beginning of June respectively. By the middle of July, all the mature population were either spent or just recovering. Records of eggs from the shore (Table 13) indicate a breeding season of about 2 months' duration from mid-April to mid-June. This agrees with the results of gonad examination, although some of the population may spawn outside this period, perhaps as late as the beginning of July.

Age at maturity

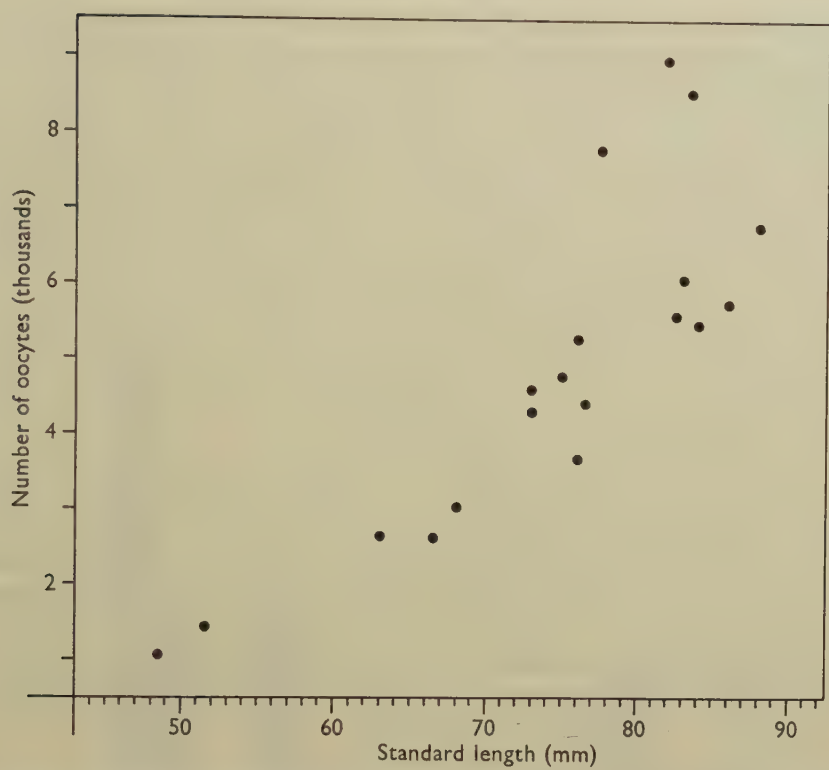
Up to the second autumn of life all fishes were immature. After the second winter, most individuals showed signs of gonad development, which, in a proportion (*ca.* 15 %) of this age-group (late 1 to early 2), was carried through to ripening and, probably, to breeding in the ensuing spring (Tables 9 and 10). At the end of the breeding season, 2-group fishes were found whose gonads had the typical spent appearance (stage V). In others, there were indications of testicular vascularization or oocyte resorption, but the overall impression was that these gonads had not passed beyond a developmental (II) or early ripening (III*a*) stage. This was more easily seen among the males than in the females. It seems, therefore, that a certain percentage of the 2-group fishes which undergo initial development of the gonads do not, in fact, ripen during the breeding season at the beginning of their third year. Other members of the same age-group (about a quarter) remain immature throughout this breeding period. By the end of the year, however, the few individuals obtained had ripening gonads, and the fishes of age-group 3 were nearly all mature (Tables 9 and 10). A few examples entering age-group 3 may still be in a developing stage (II), and the capture of a male, late developing or at best early ripening, on 1 June 1960 could mean that some of this age do not mature. All fishes in age-groups 4 and above were mature. There was no case of apparent loss in reproductive capacity with advanced age, even among the oldest fishes encountered (age-groups 8 and 10).

Length at maturity

In the spring quarter, among 3-group and older fishes, the smallest ripening male was 58·0 mm, while an early ripening female of 58·0 mm showed definite signs of having spawned that same breeding season. The two developing males from age-group 3 taken during the same period were 58·0 and 59·5 mm. The smallest ripening 2-group fishes in each sex were a male of 50·5 mm, and a female of 48·5 mm. In this age-group, ripening individuals were on average the largest fishes (Table 11).

Fecundity

Counts of ripening or ripe oocytes were made in nineteen females collected in 1959 and 1960. The method employed was to weigh the ovaries of each fish, and then a small portion of one ovary; after counting the number of such oocytes in the latter, total number in both ovaries was easily calculated. The results (Table 12, Text-fig. 10) show a relationship between length of fish and number of oocytes, which increases with body size. Considerable variation in fecundity may exist at a particular body length. A similar conclusion was reached by Johnsen (1945) in the case of the gobiid *Pomatoschistus flavescens* (Fabricius).



Text-fig. 10. Relation between standard length and number of late ripening or ripe oocytes.

Spawning and early life history

Spawning is preceded by an intensification in the external coloration of the male, which becomes very dark with a cream to reddish horizontal band along the superior edge of the first dorsal fin (Holt & Byrne, 1898, 1903, and personal observation). The breeding behaviour is not known in detail. At Poyllbrein, egg patches were found on the shore only from April to June (Table 13). The eggs were laid on the flat undersurfaces of submerged stones in pools at mid-tide level and below, being deposited in a single layer, usually close together, at an observed density of about 110 to 140 per square centimetre. In the vicinity of Naples, Lo Bianco (1909) found ova deposited on tubes of the polychaetes *Hydroides* and *Spirographis*, and also over the mantle of the tunicate *Ciona*. The eggs are fusiform, with pointed apex, and attached to the substrate by filaments arising from the region of the micropyle (Lebour, 1919, and Sparta, 1934, provide figures). Holt & Byrne (1898, 1903) stated that the eggs of this species were 1·84–1·90 mm in length. Other workers (Raffaelli, 1898; Lo Bianco, 1909; Hefford, 1910; Le Danois, 1913; Lebour, 1919; Sparta, 1934) record greater lengths from 2·0 to 3·0 mm, mostly

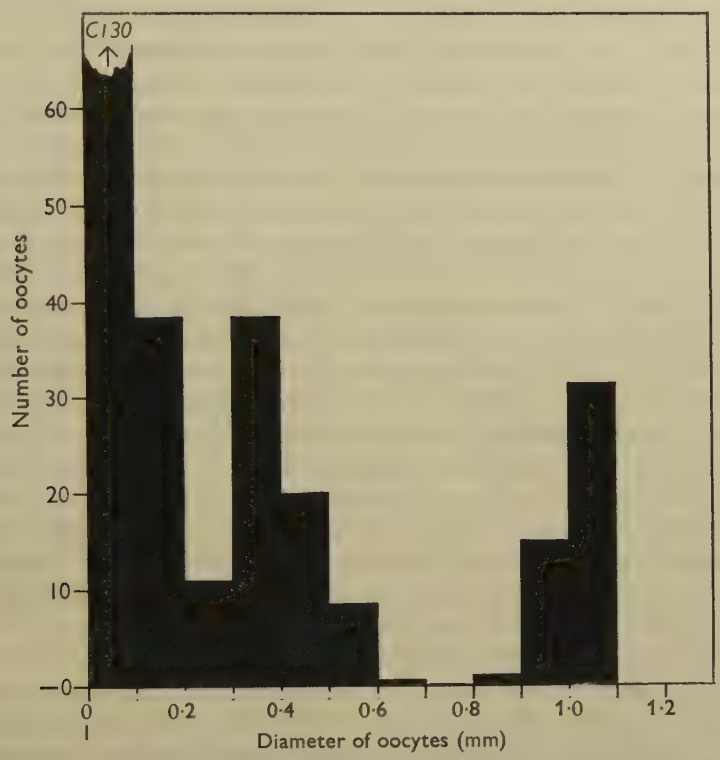
2·2–2·5 mm. Those found at Poyllbrein measured 2·4–2·6 mm in length. After spawning, the egg patch is guarded by the male parent (Holt & Byrne, 1903; Sparta, 1934). As many as nearly 7000 eggs have been found in the one patch (Table 13). This is within the number yielded by a single large female (Table 12). According to Le Danois (1913), several females may contribute towards the same egg mass which consequently may include eggs in different stages of development. Vivien (1939*b*) found that, in captivity, three females spawned with the same male in the course of 27 h, with the production of 2398 eggs. The average number per female is here much lower than is indicated by the results in Table 12; the ovaries of these fishes were, however, only partly collapsed ('dégonflé de moitié'). In all but one of the egg patches referred to in Table 13, the eggs were at roughly the same developmental stage, while in one instance the patch appeared to have been added to some days after the first spawning.

Development takes about 3 weeks (Lebour, 1919), and the young fish hatches head first from the apex of the capsule. The standard length on hatching is 3·6–3·8 mm. Total lengths mentioned by previous authors are 4·8 (Hefford, 1910), 4·0 (Lebour, 1919), and 4·76 mm (Sparta, 1934). The newly hatched fish carries a small quantity of yolk, which is quickly absorbed. Older post-larvae have been obtained from shore pools at Poyllbrein from the beginning of July, and also from inshore waters around Plymouth (Lebour, 1919). Metamorphosis occurs at a length of about 9·5–10·5 mm; young demersal individuals appear in the pools from August.

Number of broods

Hickling & Rutenberg (1936) have demonstrated that the size–frequency distribution of oocytes in the teleost ovary is related to the duration of the breeding season and to the number of spawnings within that time. The condition in *G. paganellus* is typical of that shown by species which produce more than one brood in the course of the breeding season. At the start of the spawning period, the ripe ovary contains, in addition to the ripe oocytes, another group of smaller oocytes clearly demarcated from the many tiny cells in the ovary (Text-fig. 11). The members of this group already possess yolk and show the features of those comprising the leading group in ovaries at the commencement of the early ripening (III*a*) stage. After a spawning, ripening of these provides the next batch of ripe oocytes. In the fishes taken at Poyllbrein, ripe oocytes were never found in an ovary containing other oocytes at a more advanced stage than those in the earliest ripening (III*a*) stage of the ovary. It would seem that once a female ripens, the ripe oocytes are used up rapidly, perhaps during a few days of spawning activity, so that later spawning in that same breeding season depends on the maturation of the early ripening group or others subsequently differentiated. Text-fig. 10 suggests that at least two broods of eggs are laid during the spawning

period, with ample reserves of small oocytes for at least another batch. Qasim (1956*b*) obtained three spawnings from a female *Blennius pholis* in the 6 weeks from late April to early June. This species has a rather similar size-frequency distribution of oocytes to that found in *G. paganellus* (Qasim, 1956*a*, fig. 1). At the close of the breeding season in the latter, the early ripening oocytes left after the last spawning do not undergo further ripening and, after a short time, are reabsorbed as the ovary enters stage V (spent). Vivien (1939*a*) noted a similar end to the spawning period at Wimereux.



Text-fig. 11. Size frequency of oocytes in portion of ripe ovary of *G. paganellus* at beginning of spawning period (late April).

DISCUSSION

The present species is the only one of the genus *Gobius* (sensu stricto, De Buen, 1930) whose life history has been examined in any detail. Among the other European gobies, the life histories of *Aphia minuta* (Risso) and *Crystallogobius nilssoni* (Düben & Koren) have been the subject of researches by Collett (1878), that of *Pomatoschistus minutus* by Shann (1910) and Swedmark (1958), and that of *P. flavescens* by Johnsen (1945). In the Manx area,

the last two species, together with *P. microps* (Kröyer) and *P. pictus* (Malm), have been investigated by the author (unpublished). Typically, these *Pomatoschistus* species begin to mature before reaching the age of 1 year and breed after the first winter of life. Few individuals survive a second winter, so that the maximum life span is in the region of 2 years. Collett (1878) asserted that, in Oslofjord, Norway, both *Aphia minuta* and *Crystallogobius nilssoni* were truly annual fishes, with complete mortality of the year-old adults subsequent to the breeding season. From the preceding sections of this paper, it will be realized that the life history of *G. paganellus* differs quite markedly from those exhibited by the above species in the much greater life span and by maturation in 2 years at the earliest instead of 1. There is a comparable difference in maximum size: *Aphia*, *Crystallogobius*, and the *Pomatoschistus* species do not exceed a maximum total length of about 65 mm, except for *P. minutus*, which is a more slender species reaching 95 mm, whereas *G. paganellus* attains a total length of 120 mm. Other British representatives of the same genus range from 120 to 230 mm in total length. These are *Gobius* sp. (*auratus* Risso?) (120 mm), *G. forsteri* Corbin (120 mm), *G. niger* L.(140 mm) and *G. cobitis* Pallas (230 mm), the lengths given being those for British or neighbouring waters (Marine Biological Association, 1957; Corbin, 1958; Duncker, 1928; Wheeler, 1960). It would appear likely that these larger gobies enjoy a similar longevity to that discovered in *G. paganellus*, but there is no published information on this subject. However, a small collection of *G. niger* has been available for study during the present work. These fishes were trawled in the estuary of the Roach, Essex, on 5 July 1960. An adult male of 86·0 mm standard length was found to be in age-group 4 or 5, while an adult female, 98·0 mm, was in age-group 5. The otoliths of both specimens were difficult to read, and on par with the most illegible in *G. paganellus*. The ages determined are of the same order as those found in the latter, and suggest that duration of life may be similar in the two species.

On British coasts, relative longevity and late maturation may be as characteristic of the genus *Gobius* as reduced life span and early maturation is of the *Pomatoschistus* species. There is some evidence, however, that these features may vary from south to north in the geographical range of a species. In *P. flavescens*, Johnsen (1945) discovered that the life history is typically pomatoschistoid in Danish waters, the Baltic, and the southern parts of Norway, just as it is in the Isle of Man (personal observation). Towards the north along the Norwegian coast, an increasing proportion of these fishes do not mature until after the second winter of life. At the northern limit of the species (including the Faeroes) the breeding population is composed entirely of fishes in this age-group, which die off on completion of spawning. This retardation in the attainment of sexual maturity is similar to that found in *G. paganellus* in the Isle of Man, where this species is near its northern limit (see Introduction). *G. forsteri* and *G. cobitis* reach their

limit of distribution at the British Isles (south-west coasts), and may be expected to follow *G. paganellus* in respect of age at maturity. *G. niger*, on the other hand, extends as far north as Trondheim on the coast of Norway (Duncker, 1928), and, around the British Isles, might be expected to show signs of maturation in the 1-group fishes (at about 1 year old), but examination of a few examples of this age-group from the Roach estuary in early July did not reveal other than immature gonads. A later sample would be needed for more satisfactory investigation of this question. Three of the five British *Gobius* species definitely occur in the Mediterranean; a fourth, *G. forsteri*, may be found there (Miller, 1961). It is of interest to note that Lo Bianco (1909) records *G. paganellus* breeding at Naples when 1 year old, but presents no evidence in support of this statement.

For British marine teleosts, Qasim (1956*a*) deduced the general rule that southern (mediterranean-boreal) forms breed during spring and summer, in a protracted breeding season of 5–6 months. The ovaries of these species show maturing oocytes in at least two size groups, and provide a series of batches of ripe eggs during the spawning period. In distribution, *G. paganellus* clearly belongs to the southern element of the British fish fauna; Text-fig. 11 indicates that the pattern of oocyte maturation is also typical of the latter. The length of the breeding season in *G. paganellus* is, however, only about 2 months (April to June) and is considerably shorter than those of other southern fishes investigated in British seas (Qasim, 1956*a*). In the gobies, the four *Pomatoschistus* species studied in the Manx area have a period of reproduction lasting about 5 months. As indicated by the occurrence of eggs, *G. paganellus* at Plymouth breeds during spring and early summer (Holt & Byrne, 1898) but into late summer according to Lebour (1919); at Wimereux, in the Pas de Calais, from April to June (Vivien, 1939*b*); at Roscoff (Brittany), from April (or March, Cantacuzene, 1956) to August (Le Danois, 1913); in the Mediterranean, at Venice, from February to May (Cavinato, 1950), and, at Naples, from January to June (Lo Bianco, 1909). On the basis of this information, it would appear that near the northern (Isle of Man) and north-eastern (Wimereux) limits of *G. paganellus*, the breeding season is both later in occurrence and shorter in duration than in the more southern areas of distribution, exemplified by Roscoff, Venice and Naples. Similar changes in spawning season with latitude are exhibited by other mediterranean-boreal and lusitanian-boreal teleosts (including another goby, *Pomatoschistus flavescens*) as they approach their northern limits (Table 14). Relative proximity to the northern boundary of distribution may therefore explain the differences between the spawning periods of *G. paganellus* and the *Pomatoschistus* species in the Isle of Man, since all four of the latter extend to the Norwegian coast (Duncker, 1928).

Orton (1920) suggested that 'low latitude forms in relatively high latitudes should only breed in summer', and it might be expected that *G. paganellus*

would breed in the warmest months of the year near the northern border of its range, i.e. in summer and early autumn at Poyllbrein. If this were the case, and breeding occurred in late summer or after, the fry would be subjected to declining sea temperatures during their post-larval and early demersal life (estimated as covering about 3 months from hatching). Breeding seasons may be regarded as adapted by natural selection to fit environmental conditions (Lack, 1954) and, for the present species, breeding from April to June in the Isle of Man leads to hatching of young at a period of rapidly increasing sea temperature and abundant plankton. Their subsequent growth in the post-larval condition and, after metamorphosis, as demersal individuals takes place in the warmest part of the year, when the chief growing period in older age-groups occurs (June to October). Fishes from late spawnings would encounter adverse conditions of temperature when post-larval or early demersal—particularly vulnerable stages in the life history of a fish. There would be, therefore, in this part of the species' range, selection pressure against ripening for the first time of 2-group individuals in mid-summer, as happens in *Blennius pholis* in the Irish Sea (Qasim, 1957), and against any prolongation of spawning by older age-groups.

In *G. paganellus*, the breeding season is closed by resorption of early ripening oocytes in mature or maturing females, followed by regressive changes in the testes of the males. Ripening oocytes are resorbed in female *G. paganellus* as a result of hypophysectomy (Vivien, 1939*a*, 1941), and, in the wild, the same process may be initiated by a drop in pituitary gonadotrophin levels. Seasonal fluctuation in gonadotrophic activity is known among teleosts, and is probably related to variation in the external environment (Pickford & Atz, 1957). Examination of sections has shown that resorption of a ripening oocyte in this species is characterized by the formation of a typical 'pre-ovulatory corpus luteum' (as described by Bretschneider & Duyvene de Wit, 1947). Ball (1960) concluded that such bodies in the teleost ovary secrete steroid sex hormone. In *G. paganellus*, where they apparently occur only at the end of the breeding season after sexual activity is over, an endocrine function of this nature would seem doubtful. However, secretion at this time may promote meiotic division of the germ cells and accelerate the formation of oocytes from the germinal epithelium as a first preparation of the ovary for the next breeding season. The influence of hormones on this process in fishes has been reviewed by Ball (1960).

I wish to thank Mr J. S. Colman and Mr A. B. Bowers for helpful criticism of this paper. I am also grateful to Miss M. H. Baucher for assistance in collecting, to Dr J. W. Jones who suggested the use of Liquid Derris as a fish poison, to Mr A. L. Rice for information on the diet of *Acanthocottus bubalis*, and to Mr A. C. Wheeler for specimens of *Gobius niger*. This work was carried out during the tenure of a D.S.I.R. Research Studentship.

SUMMARY

Age, growth and reproduction of the intertidal teleost, *Gobius paganellus*, has been investigated in the Isle of Man from March 1959 to September 1960. Samples were obtained by poisoning shore pools with a rotenone emulsion.

Age was determined by examination of otoliths where a translucent annual ring is laid down in spring. Although the potential life span is at least 10 years, immature fishes of the first two age-groups constitute most of the population. An onshore breeding migration is postulated. An empirical survival curve indicates a high mortality rate during the first two years of life. Instances of predation on *G. paganellus* are given.

Growth in length is logarithmic, with marked decrease in growth rate from about the age of sexual maturity. During the year, growth occurred from June to October. Maximum standard lengths recorded were 92·5 mm (female) and 90·0 mm (male).

The overall sex ratio of females to males was 1:1·326; this predominance of males persisted through the age-groups. The reproductive organs are briefly described, and a classification of gonad maturation is proposed. The breeding season lasted from mid-April to mid-June. Sexual maturity was reached at the end of the second or third year of life. Smallest ripening individuals found were 48·5 mm (female) and 50·5 (male). Fecundity increased with body size. Eggs were deposited on stones in shore pools and guarded by the male. Post-larvae occurred in pools from the beginning of July, and the first demersal individuals from August. Size–frequency distribution of oocytes in the ovary indicates that at least two broods may be produced in a single breeding season.

In maximum size, longevity, and age at maturity, *G. paganellus* differs from other European gobies so far studied. It is suggested that a similar life history is exhibited by other British species of *Gobius*. The short breeding season of *G. paganellus* in the Isle of Man is held to be a result of proximity to the northern limit of distribution of this species. Examples of this phenomenon in other teleosts are provided. The significance of pre-ovulatory corpora lutea in the ovary of *G. paganellus* is briefly discussed.

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TABLE 1. NUMBER OF FISHES WITH OPAQUE OR TRANSLUCENT EDGES TO OTOLITHS IN EACH COLLECTING PERIOD

Collecting period (1959)	Opaque	Transl.	Collecting period (1960)	Opaque	Transl.
Mar./Apr.	65	2	Jan./Feb.	97	0
Late Apr.	19	14	Feb./Mar.	54	3
May/June	45	41	Mar./Apr.	94	18
June/July	60	5	Late Apr.	12	35
July/Aug.	88	0	May/June	29	55
Aug./Sept.	50	0	June/July	72	3
Late Sept.	68	0	July/Aug.	73	0
Late Oct.	55	1	Aug./Sept.	33	0
Nov./Dec.	46	0			
Late Dec.	7	0			

TABLE 2. NUMBER OF FISHES WITH WIDE OR NARROW OPAQUE EDGES TO OTOLITHS FROM LATE WINTER TO EARLY SUMMER

Former category includes fishes without translucent rings in otoliths.

Collecting period (1959)	Wide	Narrow	Collecting period (1960)	Wide	Narrow
Mar./Apr.	65	0	Feb./Mar.	54	0
Late Apr.	19	0	Mar./Apr.	94	0
May/June	16	29	Late Apr.	12	0
June/July	2	54	May/June	1	28
			June/July	1	71

TABLE 3. NUMBER OF FISHES IN EACH AGE-GROUP PER QUARTER

	Total no.	Age-groups 0	1	2	3	4	5	6	7	8	9	10
Spring*	470	—	297	89	27	11	21	20	3	2	—	—
Summer*	490	108	244	67	21	18	23	8	—	—	—	1
Autumn	266	220	33	4	—	2	3	4	—	—	—	—
Winter	185	107	36	11	10	10	7	3	1	—	—	—

*Numbers for 1959 and 1960 combined.

TABLE 4. MEAN LENGTH IN EACH QUARTER

Age-group	Quarter	Males No.	Males Mean length (mm)	Females No.	Females Mean length (mm)	Overall length range (mm)
0	Summer	Unsexed:		120	14·16	9·5–27·0
	Autumn	124	23·55	79	24·46	13·5–35·0
	Winter	62	26·36	41	25·77	17·0–36·0
1	Spring	159	27·43	132	27·37	15·5–38·0
	Summer	141	41·16	97	40·85	23·5–56·0
	Autumn	18	48·47	15	51·16	40·0–59·0
	Winter	20	51·54	16	49·67	39·0–59·0
2	Spring	59	49·39	39	49·66	37·0–63·0
	Summer	38	61·72	29	60·12	50·0–72·0
	Autumn	2	74·00	3	70·25	66·5–75·5
	Winter	6	68·22	6	63·73	57·0–75·0
3	Spring	16	63·93	11	62·01	55·0–75·0
	Summer	10	70·00	10	68·19	62·0–77·5
	Autumn	No sample		No sample		
	Winter	6	74·35	4	67·50	64·0–78·0
4	Spring	3	68·75	8	67·27	63·0–74·0
	Summer	10	77·71	8	75·01	69·0–87·0
	Autumn	2	80·50	No sample		78·0–83·0
	Winter	5	77·00	5	72·80	70·0–80·0
5	Spring	14	77·07	7	77·42	69·5–82·5
	Summer	12	78·08	11	82·69	72·0–90·0
	Autumn	No sample		3	83·67	81·0–86·0
	Winter	5	82·38	2	84·00	81·0–90·0
6	Spring	12	82·03	8	78·52	75·0–85·0
	Summer	3	79·50	5	87·44	77·5–92·5
	Autumn	No sample		4	85·88	80·0–91·0
	Winter	1	81·00	2	85·00	81·0–88·0
7	Spring	2	87·00	1	82·50	82·5–89·0
	Winter	No sample		1	83·00	
8	Spring	1	88·00	1	76·00	76·0–88·0
10	Summer	No sample		1	92·00	

TABLE 5. MONTHLY MEAN LENGTHS FOR YEAR-CLASSES 1957 TO 1960 FROM MARCH 1959 TO SEPTEMBER 1960

Age-group	Collecting period	Males No.	Males Mean length (mm)	Females No.	Females Mean length (mm)	Overall length range (mm)
Year-class 1957						
2	Mar./Apr.	3	43·17	4	46·25	37·0–50·0
	Late Apr.	1	51·00	1	50·00	50·0–51·0
	May/June	8	52·50	4	50·13	43·0–60·0
	Late June	2	56·00	6	55·67	51·0–62·5
	July/Aug.	8	61·56	9	58·22	53·0–67·0
	Aug./Sept.	2	66·00	3	63·33	61·0–68·0
	Late Sept.	2	74·00	1	66·50	66·5–75·5
	Late Oct.	No sample		No sample		
	Nov./Dec.	No sample		1	74·00	—
	Late Dec.	2	63·00	1	57·00	57·0–63·0
	Jan./Feb.	3	66·67	3	67·68	62·0–73·0
	Feb./Mar.	1	75·00	1	66·50	66·5–75·0
Year-class 1958						
1	Mar./Apr.	30	22·60	20	23·00	15·5–30·0
	Late Apr.	14	28·54	10	26·85	19·0–33·0
	May/June	36	28·18	28	28·46	19·0–38·0
	Late June	28	31·88	21	32·93	23·5–41·5
	July/Aug.	31	40·94	20	40·45	31·0–50·0
	Aug./Sept.	19	46·97	15	47·80	38·5–54·0
	Late Sept.	12	53·50	9	51·67	40·5–58·0
	Late Oct.	5	46·90	5	42·80	40·0–56·5
	Nov./Dec.	1	45·00	1	59·00	45·0–59·0
	Late Dec.	No sample		No sample		
	Jan./Feb.	9	50·72	12	50·71	39·0–59·0
	Feb./Mar.	11	52·36	4	48·62	43·0–58·0
2	Mar./Apr.	18	48·17	14	48·07	41·0–63·0
	Late Apr.	9	47·89	4	51·86	40·0–60·0
	May/June	20	53·60	12	51·67	45·0–59·0
	June/July	13	58·62	4	56·88	50·0–65·0
	July/Aug.	8	60·06	5	62·10	52·5–66·5
	Aug./Sept.	5	68·00	2	64·50	57·0–72·0
Year-class 1959						
0	Late June	Unsexed:		1	9·00	—
	July/Aug.	Unsexed:		21	10·43	9·5–14·0
	Aug./Sept.	Unsexed:		60	15·18	9·5–27·0
	Late Sept.	43	23·40	24	22·94	13·5–35·0
	Late Oct.	52	22·40	40	24·03	16·0–31·0
	Nov./Dec.	29	24·84	15	26·40	17·0–34·0
	Late Dec.	1	27·00	2	25·75	19·5–32·0
	Jan./Feb.	42	24·55	21	25·45	17·0–36·0
	Feb./Mar.	19	27·53	18	26·11	18·0–36·0
1	Mar./Apr.	50	26·68	44	27·72	19·5–35·0
	Late Apr.	13	28·58	11	27·55	20·0–33·0
	May/June	16	30·00	19	30·63	19·5–37·0
	June/July	25	38·20	15	35·43	30·0–45·0
	July/Aug.	27	41·00	15	40·93	33·0–47·0
	Aug./Sept.	11	47·95	11	47·55	40·0–56·0
Year-class 1960						
0	Aug./Sept.	Unsexed:		38	16·78	9·5–26·0

TABLE 6. NUMBER AND PERCENTAGE OF EACH SEX PER AGE-GROUP

	Total	Age-groups 0*	1	2	3	4–10
No. of males	724	186	338	96	32	72
No. of females	546	120	260	75	26	65
% males	57·0	60·8	56·5	56·1	55·2	52·6
% females	43·0	39·2	43·5	43·9	44·8	47·4

* Autumn and winter quarters only.

TABLE 7. NUMBER OF MALES (AGE-GROUP 3 AND OLDER) PER MATURITY STAGE

Collecting period	Stages II	III	IV	V	Total no.
Mar./Apr.	1	3	15	—	19
Late Apr.	—	—	9	—	9
May/June	1	—	13	6	20
June/July	—	—	2	7	9
July/Aug.	9	—	—	11	20
Aug./Sept.	4	—	—	2	6
Late Sept.	No sample				
Late Oct.	—	1	—	—	1
Nov./Dec.	—	1	—	—	1
Late Dec.	—	1	—	—	1
Jan./Feb.	—	9	9	—	18
Feb./Mar.	—	1	—	—	1

TABLE 8. NUMBER OF FEMALES (AGE-GROUP 3 AND OLDER) PER MATURITY STAGE

Collecting period	Stages II	III*a*	III*b*	IV	V	Total no.
Mar./Apr.	—	6	11	—	—	17
Late Apr.	—	1	3	3	—	7
May/June	—	7	3	—	2	12
June/July	9	—	1	—	5	15
July/Aug.	10	—	—	—	3	13
Aug./Sept.	8	—	—	—	—	8
Late Sept.	5	—	—	—	—	5
Late Oct.	No sample					
Nov./Dec.	—	2	—	—	—	2
Late Dec.	No sample					
Jan./Feb.	—	9	—	—	—	9
Feb./Mar.	—	3	2	—	—	5

TABLE 9. NUMBER OF MALES PER MATURITY STAGE IN EACH COLLECTING PERIOD

Autumn of age-group 1 to beginning of age-group 3.

Collecting period	Stages I	II	III	IV	V and other resorb.	Total no.
Late Sept.	9	3	—	—	—	12
Late Oct.	3	2	—	—	—	5
Nov./Dec.	1	—	—	—	—	1
Late Dec.	No sample					
Jan./Feb.	1	8	—	—	—	9
Feb./Mar.	1	10	—	—	—	11
Mar./Apr.	8	11	1	1	—	21
Late Apr.	2	8	—	—	—	10
May/June	7	18	2	—	1	28
June/July	4	7	—	—	4	15
July/Aug.	3	7	—	—	6	16
Aug./Sept.	—	7	—	—	—	7
Late Sept.	—	2	—	—	—	2
Late Oct./Dec.	No sample					
Late Dec.	—	—	2	—	—	2
Jan./Feb.	—	—	3	—	—	3
Feb./Mar.	—	—	—	1	—	1
Mar./Apr.	—	1	3	3	—	7

TABLE 10. NUMBER OF FEMALES PER MATURITY STAGE IN EACH COLLECTING PERIOD

Autumn of age-group 1 to beginning of age-group 3.

Collecting period	Stages I	II	III*a*	III*b*	IV	V and other resorb.	Total no.
Late Sept.	9	—	—	—	—	—	9
Late Oct.	5	—	—	—	—	—	5
Nov./Dec.	1	—	—	—	—	—	1
Late Dec.	No sample						
Jan./Feb.	1	10	—	—	—	—	11
Feb./Mar.	3	2	—	—	—	—	5
Mar./Apr.	3	11	4	—	—	—	18
Late Apr.	2	1	1	1	—	—	5
May/June	4	6	3	1	—	2	16
June/July	1	5	—	—	—	4	10
July/Aug.	4	10	—	—	—	—	14
Aug./Sept.	1	4	—	—	—	—	5
Late Sept.	—	1	—	—	—	—	1
Late Oct.	No sample						
Nov./Dec.	—	—	1	—	—	—	1
Late Dec.	—	—	1	—	—	—	1
Jan./Feb.	—	—	3	—	—	—	3
Feb./Mar.	—	—	1	—	—	—	1
Mar./Apr.	—	—	3	—	—	—	3

TABLE 11. MEAN LENGTHS AND LENGTH RANGE IN AGE-GROUP 2 FOR MATURITY STAGES DURING SPRING (1959 AND 1960)

Number of individuals in parentheses.

	Maturity stages					
	I		II		III and IV	
	Mean length (mm)	Range (mm)	Mean length (mm)	Range (mm)	Mean length (mm)	Range (mm)
Males	45·79 (17)	37·0–55·0	51·61 (37)	43·0–60·0	56·25 (4)	50·5–63·0
Females	46·78 (9)	41·0–53·5	49·06 (18)	41·5–58·0	52·75 (10)	48·5–60·0

TABLE 12. NUMBER OF RIPENING OR RIPE OOCYTES IN FEMALE *GOBIUS PAGANELLUS* (1959 AND 1960)

Date	Standard length (mm)	Maturity stage	Calculated no. of oocytes
8 Feb.	83·0	Late III*a*	6064
8 Feb.	86·0	Late III*a*	5730
24 Feb.	82·0	III*b*	8978
24 Feb.	88·0	Late III*a*	6750
22 Mar.	73·0	III*b*	4597
22 Mar.	75·0	III*b*	4776
22 Mar.	76·0	III*b*	5265
22 Mar.	82·5	III*b*	5571
22 Mar.	83·5	III*b*	8546
23 Mar.	66·5	III*b*	2628
24 Mar.	51·5	Late III*a*	1425
24 Mar.	68·0	III*b*	3030
24 Mar.	73·0	III*b*	4305
24 Mar.	77·5	III*b*	7786
21 Apr.	63·0	V	2642
22 Apr.	84·0	V	5460
20 May	48·5	Late III*a*	1054
20 May	76·5	III*b*	4418
31 May	76·0	III*b*	3682

TABLE 13. EGG PATCHES FOUND ON THE SHORE DURING 1959 AND 1960

Date	Estimated no. of eggs*	Developmental stage†	Estimated days in development‡	Estimated date of laying
29 Apr.	—	Early	7–8	22 Apr.
19 May	—	Late	*ca.* 17	*ca.* 2 May
20 May	1650	Early	*ca.* 6	*ca.* 14 May
1 June	6750	Late	17–18	15 May
1 June	3900	Early	5–7	26 May
1 June	5500	Early	2	30 May
1 June	2800	Late	16–17	16 May
1 June	—	Hatched	*ca.* 20	*ca.* 12 May
2 June	4500	Early	1–3	31 May
30 June	4100	Hatching	*ca.* 20	*ca.* 10 June

* From number per cm^2 determined for that patch.

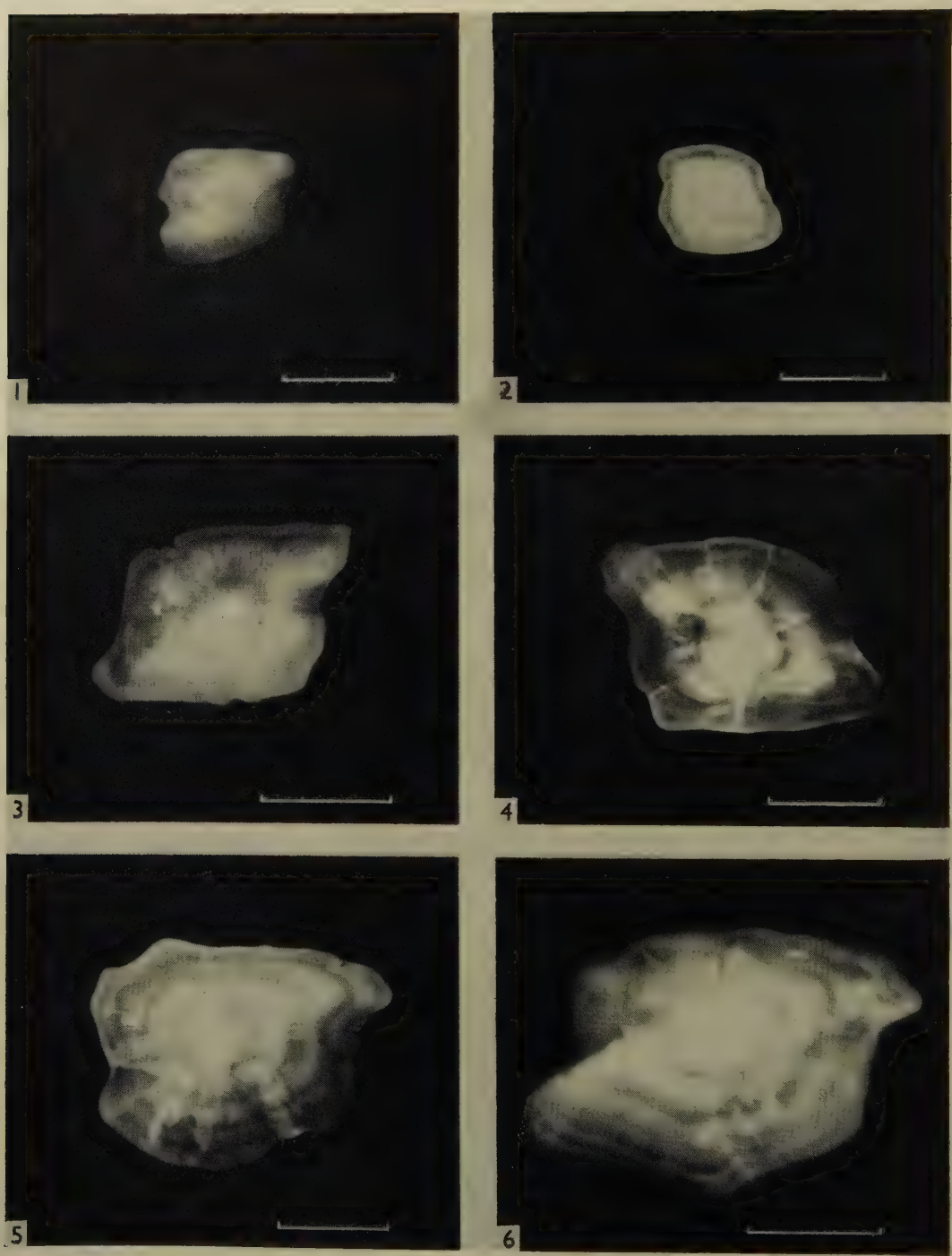
† Early: without eye pigmentation. Late: eye conspicuously pigmented, to hatching.

‡ Derived from Lebour (1919).

TABLE 14. DELAY IN ONSET AND SHORTENING OF SPAWNING PERIOD WITH APPROACH TO NORTHERN LIMITS OF BREEDING RANGE

Mediterranean-boreal and lusitanian-boreal (*) species.

Species	Area	Latitude ° N.	Duration of spawning period	No. of months	Spawning period assessed by occurrence of	Authority
Clupea sprattus L.	Middle Baltic	55–57	End of May–end of July	2	Ripe gonads	Mulicki, 1948
	Plymouth	50	January–July	7	Eggs	Marine Biological Association, 1957
	Mediterranean	32–44	Dec.–Mar.	4	Eggs	Fage, 1920
C. pilchardus Walbaum	S. North Sea	51–53	(May) June–July (Aug.)	2–4	Eggs	Furnestin, 1939; Cushing, 1957
	Plymouth	50	All year	12	Eggs	Marine Biological Association, 1957
	Mediterranean	32–44	Nov.–June	8	Larvae	Fage, 1920
Stolephorus encrasicolus (Lac.)	S. North Sea	52–54	End of May–end of July	2	Eggs	Fage, 1920; Ehrenbaum, 1936
	Mediterranean	32–44	Mid-Apr.–mid-Sept.	5	Eggs and larvae	Fage, 1920
Gadus merlangus L.*	S.W. Iceland	63–65	Mid-Apr.–mid-July	3	Ripe gonads and eggs	Schmidt, 1909; Saemundson, 1949
	Isle of Man	54	Feb.–June	5	Ripe gonads	Bowers, 1954
Merluccius merluccius (L.)	Skagerak	57–59	July–Aug, or Aug.	2 or 1	Larvae	Schmidt, 1909; Tåning, 1938
	S.W. Ireland	50–53	Apr.–July	4	Ripe gonads	Hickling, 1930
	Mediterranean (Naples)	41	Jan.–June	6	Ripe gonads	Lo Bianco, 1909
Scomber scombrus L.	Skagerak	57–59	End of May–June (July?)	1 (2?)	Eggs	Dannevig, 1947
	Celtic Sea	48–52	Mid-Mar.–end of July	4½	Eggs	Corbin, 1947
	Mediterranean	32–44	Dec.–Apr.	5	Eggs	Padoa, 1937
Trachurus trachurus (L.)	S. North Sea	52–54	June–Aug.	3	Eggs	Ehrenbaum, 1905
	Plymouth	50	June–July	2	Eggs	Marine Biological Association, 1957
	Mediterranean	32–44	All year	12	?	Padoa, 1937
Gobius paganellus L.	Isle of Man	54	Mid-Apr.–Mid-June	2	Eggs	Personal observation
	Roscoff	49	Mar.–Aug.	6	Eggs	Le Danois, 1913; Cantacuzene, 1956
	Mediterranean (Naples)	41	Jan.–June	6	Eggs	Lo Bianco, 1909
Pomatoschistus flavescens (Fabr.)*	Faeroes	62	May–July	3	Ripe gonads	Tåning, 1940
	Isle of Man	54	Mid-Mar.–beginning of Aug.	4½ +	Eggs and ripe gonads	Personal observation



(*Facing p.* 769)

EXPLANATION OF PLATE I

Otoliths of *Gobius paganellus*, after clearing in creosote.

Fig. 1. Male, 30·0 mm, 8 April 1960, lacking annual rings.
Fig. 2. Female, 31·0 mm, 28 June 1960, one ring and narrow opaque edge.
Fig. 3. Male, 59·0 mm, 24 July 1959, two rings and narrow opaque edge.
Fig. 4. Male, 66·0 mm, 22 March 1960, two rings and wide opaque edge.
Fig. 5. Male, 72·0 mm, 27 March 1960, four rings and wide opaque edge.
Fig. 6. Female, 83·0 mm, 1 August 1960, six rings and narrow opaque edge.

Scale = 1 mm.

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THE SPAWNING OF *ARENICOLA MARINA* (L.)

III. MATURATION AND SHEDDING OF THE OVA

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(Text-fig. 1)

The breeding season of the lugworm (*Arenicola marina* L.) coincides with, or is preceded by, the first major fall in air temperatures to occur (at low tide) during the autumn (Howie, 1959). This fall in temperature may provide the environmental spawning stimulus, although Duncan (1960) favours a stimulus associated with tidal phases. More recently it has been found that male lugworm can be induced to spawn by injecting into the body cavity tissue extracts of whole worms; the spawning stimulant being located in the lipid fraction of these extracts (Howie, 1961). In the lugworm the gametes develop freely in the body cavity over a period of months (Ashworth, 1904; Newell, 1948) during which time they must be rejected by the ciliated funnels of the nephromixia, which act as gonoducts. Tissue extracts appeared to induce spawning primarily by bringing about a breakdown of the sperm morulae (plates) to free spermatozoa in the coelom, it being suggested that whereas the former are rejected by the nephromixia the latter are accepted and shed. This has subsequently been confirmed (unpublished work). In the work described below attention has been focused on what appeared to be a close relationship between shedding and the onset of maturation of the ova. Although Child (1898) gave an account of maturation and fertilization of eggs taken from the coelom, supposedly of *A. marina*, numerous authors have reported subsequently that coelomic eggs are not fertilizable (see Newell, 1948, for a review). It has been pointed out that Child's work was published prior to important revisions in the diagnostic features of the genus. Pirlot (1933) observed maturation of the ova occurring in the nephromixia during oviposition and concluded that, for this reason, the eggs could not be fertilized until they had been shed. Newell (1948), although bringing about fertilizations of coelomic eggs, found that development did not go beyond the 4-cell stage. The inference from this work is that maturation of the eggs succeeds the onset of shedding so that the eggs are not physiologically ripe, i.e. fertilizable and capable of normal development, until they are shed. The relationship between the onset of maturation and the onset of shedding is re-examined below. Experiments are also described in which tissue extracts were administered to female worms in an attempt to induce shedding.

ARTIFICIAL FERTILIZATIONS OF COELOMIC EGGS

To ensure that eggs of the lugworm never undergo maturation while remaining in the coelom, artificial fertilizations were attempted with these eggs, similar to those described in the literature. Eggs were taken from the body cavities of freshly dug worms by means of a glass pipette or tuberculin syringe. They were washed by decanting in Berkefeld filtered sea water and kept (at 15° C) in large beakers of filtered sea water. If spawned spermatozoa were not available coelomic spermatozoa were added to the egg suspension. Difficulty was experienced in obtaining active suspensions of coelomic spermatozoa. If, however, this sperm was allowed to remain in a virtually dry condition for 2–3 h after removal from the body cavity and then diluted in sea water a thin suspension of free spermatozoa was usually obtained. Alternatively, spermatozoa were activated in an alkaline medium (see Gray, 1920;

TABLE 1. EXPERIMENTS WITH SPAWNED AND COELOMIC OOCYTES

	Fertilizations attempted	Sperm used	development %	Max. stage of development
Coelomic eggs	33	Coelomic	(Max.) 15	8 cells
	8	Spawned	(Max.) 15	16 cells
Spawned eggs	5	Coelomic	(Av.) 86	Hatch of larvae*
	8	Spawned	(Av.) 83	Hatch of larvae
Coelomic eggs (spent worms)	2	Spawned	(Max.) 28	One larva
Coelomic eggs (partially spent worms)	3	Spawned	(Max.) 17	Hatch of larvae

* In one culture only.

Carter, 1931; Pirlot, 1933), 1 ml. coelomic sperm being diluted in 50 ml. sea water containing 1·0 ml. N/10 NaOH. After 10 min. the alkali was decanted off and the spermatozoa diluted in ordinary sea water. A few drops of the active sperm suspension were added to the eggs. Eighteen artificial fertilizations were attempted immediately prior to the breeding season and twenty-three during the breeding season.

In one experiment using coelomic spermatozoa 15% of the eggs segmented, but development did not proceed beyond the 8-cell stage. In the remainder less than 1% of the eggs divided. The results did not differ materially if naturally spawned spermatozoa were used to make the fertilizations (Table 1). In one instance a few eggs developed to the 16-cell stage. For purposes of comparison results of fertilizations attempted with spawned ova under exactly similar conditions are included in Table 1. It will be noted that the use of coelomic spermatozoa activated by alkali in some of these experiments, in no way inhibited fertilization (average development 86%) but the chances of normal development were reduced. Only one out of five cultures in which this type of sperm was used produced a hatch of larvae. A hatch of normal

larvae was produced in each of eight fertilizations using both spawned sperm and spawned eggs. As attempts to fertilize coelomic eggs were continued into the breeding season, which is of short duration, it would seem that some of the eggs used in these experiments were as ripe as possible. The results, therefore, appeared to confirm that the eggs of the lugworm never become physiologically ripe while in the coelom.

EXPERIMENTS WITH COELOMIC EGGS FROM SPENT OR PARTIALLY SPENT WORMS

A further examination of this problem was made possible by keeping a number of females in the laboratory until they spawned of their own accord. Artificial fertilizations (under similar conditions to those described above) were then attempted with eggs remaining in the body cavities of spent or partially spent worms.

Two fertilizations were carried out using spawned sperm and a small number of eggs which remained in four spent females. In the first of these experiments, 28% of the eggs reached an advanced stage of division (in excess of 32 cells) but the embryos failed to hatch. In the second fertilization there was a smaller percentage development (4·5%) but in this case a number of embryos attained a ciliated condition. One larva hatched and survived for 9 days. Subsequently three fertilizations were carried out with eggs taken from the coelom of partially spawned females. Spawned sperm obtained from males injected with tissue extracts was used in making these fertilizations. Between 8% and 17% of the embryos reached an advanced stage of division and more than a hundred larvae hatched in these three cultures (Table 1). Twenty-three larvae survived to the first chaetiger stage and were still alive a month after the fertilizations were made. The results with eggs from partly spent females were therefore markedly better than those with eggs from spent worms. As the latter had not been shed when spawning was completed it may be that they were in some respect abnormal. Although there was still some discrepancy between results with naturally spawned eggs and eggs from spent or partially spent worms (Table 1), there is no doubt that ripening is at least initiated in the body cavity. In view of the results of fertilizations with coelomic eggs from non-spawning worms already described, which were entirely unsuccessful, it appeared at this stage that while ripening is initiated in the body cavity it may not commence until after the onset of shedding.

ARTIFICIAL FERTILIZATIONS OF LATE COELOMIC EGGS

As will be seen below, in experiments in which tissue extracts were administered to female worms it was the practice to take some eggs from the body cavity some hours after the injection of extract and an attempt was made to fertilize these artificially. On one occasion some females were kept in the laboratory (8–11 days) until the end of the breeding season to ensure that the

gametes were as ripe as possible, and in this case fertilizations of coelomic eggs were attempted both before and after injection of tissue extracts. In three fertilizations with eggs taken from the body cavity *before* any form of treatment, development occurred to produce hatches of larvae which survived beyond the first chaetiger stage and appeared normal (early development is described by Newell, 1948). Two of the worms from which these eggs were taken (numbers 1 and 2, Table 2) spawned within 12 h of the fertilizations being made, the third worm failed to spawn. The results of these fertilizations and of fertilizations made once the worms had commenced to spawn were apparently much the same (Table 2). From this it appears that despite all previous unsuccessful attempts to fertilize coelomic eggs reported here and in the literature, not only do the eggs become ripe in the body cavity but in addition the onset of maturation precedes the onset of shedding.

TABLE 2. EXPERIMENTS WITH COELOMIC EGGS FROM WORMS KEPT IN THE LABORATORY (8–11 DAYS) UNTIL THE END OF THE BREEDING SEASON

	Fertilizations made *before* injection of extract		Fertilizations made *after* injection of extract	
	Development (%)	Hatch	Development (%)	Hatch
Worm 1	100	Large	95	Large
Worm 2	39*	Small*	88	Large
Worm 3	23	Small	—	—

* Included eggs from a worm which subsequently failed to spawn.

MATURATION OF THE OOCYTES

There is some difference of opinion as to the normal appearance of coelomic eggs from a non-spawning worm. Newell (1948) describes them as being 'very slightly biconvex or even biconcave...disks.' Ashworth (1904) shows them as strongly biconvex disks. This is a matter of some importance for, as will be seen below, a change of shape is a feature associated with maturation. Coelomic eggs from worms collected at Dublin during the breeding season are superficially planoconvex with a greater diameter of 180–185 μ and a lesser diameter of 120–130 μ. The plane surface may bear a wide, shallow depression about 90 μ in diameter. The nucleus is very transparent (Fig. 1 A, B). Fertilizable eggs taken from the coelom of partly spawned worms and eggs which are newly shed are similar to one another in appearance but show marked differences from those just described. The dimensions show little change but the shape is now biconvex. A deep indentation to one side of the egg (47–60 μ in diameter) is always apparent. More striking are changes in the nucleus. The nuclear area is smaller and much less transparent with a more irregular margin (Fig. 1 D, E). Subsequent to these observations on live eggs samples of fertilizable coelomic eggs from partly spent worms were

fixed simultaneously with eggs taken from the nephridia and eggs which had been newly spawned. For purposes of comparison coelomic eggs taken from a non-spawning worm were also fixed. The eggs were fixed in Sanfelice's fixative or alternatively, formal-calcium (Baker, 1944), pre-stained in eosin and embedded in paraffin wax. Sections were stained in Heidenhain's iron alum haematoxylin. The appearance of the eggs in section largely confirmed the observations on live eggs outlined above. Sections of coelomic eggs from non-spawning worms showed the disk-like shape, although no indentation was

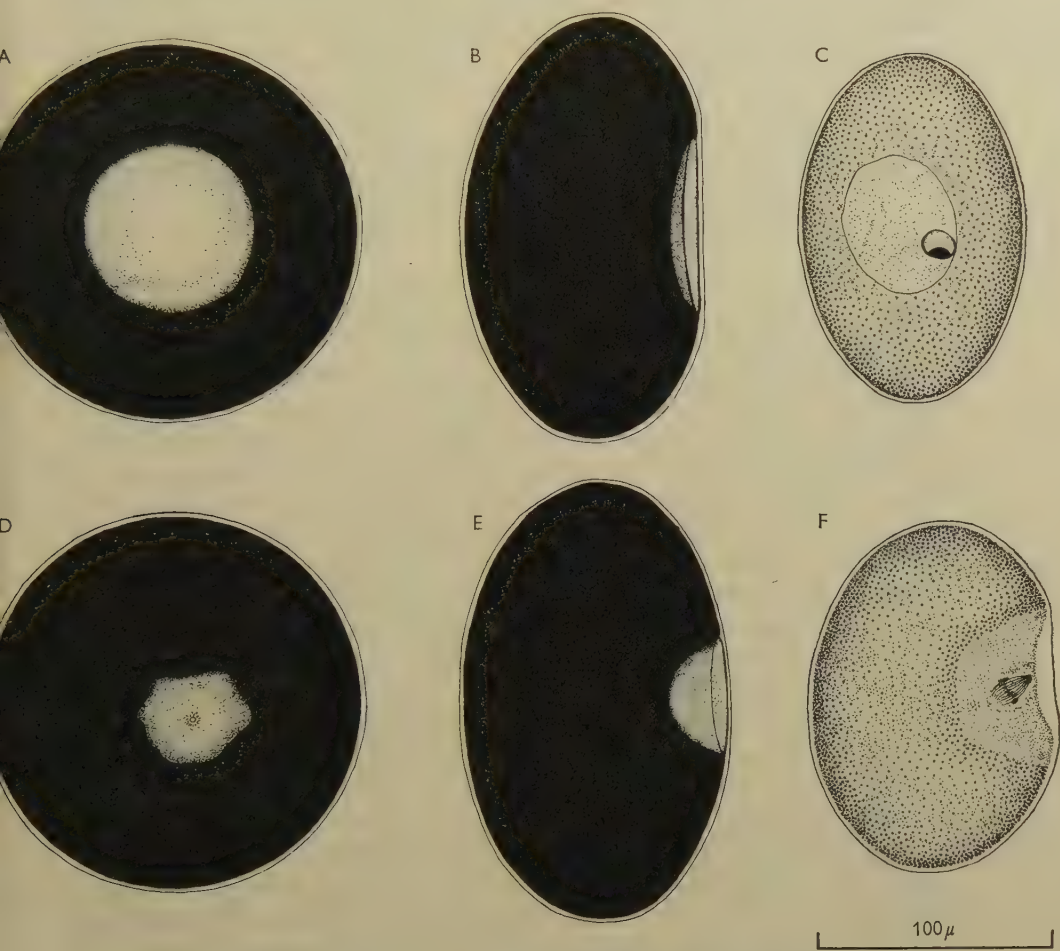

Fig. 1. The microscopic appearance of coelomic eggs from non-spawing worms (A, B and C) and spawning worms (D, E and F). A and D the greater diameter of the whole egg; B and E eggs on their sides; C and F sections through the smaller diameter. Note in C the central nucleus with prominent nucleolus. In F the nuclear area surrounds the depression to one side of the egg and a metaphase figure is present.

apparent on the plane surface. The clear nucleus, centrally situated, contained a prominent nucleolus (Fig. 1 C). In the sections of coelomic eggs from partly spent worms, eggs from the nephromixia of these worms and newly spawned eggs, there was a marked change in the location of the nuclear area which was found to surround the indentation to one side of the egg (Fig. 1 F). The denser appearance of the nuclear area observed in live eggs was due to the onset of meiosis. Metaphase figures could be seen in serial sections of almost all these eggs. Less than 1 in 500 of the eggs failed to show these features. This histological examination showed: (1) that the conclusion reached experimentally, i.e. that maturation is initiated in the body cavity, is correct: and (2) that the changes in the appearance of live eggs described above are associated with maturation so that they can be used as criteria for the recognition of ripe eggs of this species. Finally, as eggs taken from the coelom and nephromixia of a spawner and newly spawned eggs were all at the same stage of meiosis it would seem that maturation is induced in all the eggs simultaneously and that spawning follows immediately upon the onset of maturation.

THE ROLE OF MATURATION IN SHEDDING

The fact that the gametes of the lugworm develop in the body cavity over a period of months during which time they must be rejected by the ciliated funnels of the nephromixia has already been referred to. It has been shown that in male worms a change in the nature of the gametes in the body cavity (i.e. from sperm morulae to free spermatozoa) causes them to be taken up by the nephromixia so that shedding automatically ensues (Howie, 1961, and unpublished data). In view of the observation that the onset of maturation of the eggs of the lugworm immediately precedes shedding, it seemed possible that changes in the eggs at maturation already described might be all that is required to induce shedding of females. This hypothesis was tested in an experiment in which eggs were collected from dishes containing two females which had spawned in the laboratory. These spawned eggs were concentrated to give 2 ml. of eggs. One ml. of this egg suspension was injected into the body cavities of each of two non-spawning females which of course already contained immature eggs. Within 15–20 min. both of these worms began to shed eggs from the nephromixia. Large puffs of eggs were being shed after 50 min. and shedding continued for at least 5 h. Only mature eggs were shed, there being no evidence that the worms spawned any of their own complement of immature eggs. From subsequent examination of the coelomic fluid of these worms it appeared that few mature ova remained in the body cavity, although only 1 ml. of spawned eggs could be recovered the following day from the dishes in which the worms were kept. In a converse experiment immature coelomic eggs were injected into the body cavity of a spent

worm and no spawning ensued. Thus it would appear that maturation of the eggs in the body cavity is sufficient to induce the shedding of female worms.

ARTIFICIAL STIMULATION OF SPAWNING OF FEMALE WORMS

As indicated at the beginning of this paper an attempt has been made to stimulate the spawning of female worms by the technique of injecting tissue extracts which has proved successful in stimulating the spawning of males. Tissue extracts were prepared by emulsifying samples of male and female lugworm in a macerator for about 10 min. The emulsion was then lightly centrifuged to bring down the sand present and the mother liquor was filtered through Whatman no. 1 filter paper. 0·5 ml. of the filtrate (crude extract) was injected or, alternatively, the emulsion was extracted by alcohol, ether or benzene and the solute which was recovered was injected using 0·5 ml. of 0·5 M saline or a similar volume of arachis oil as carriers. The concentration of the extracted fat injected was similar to that contained in the original emulsion (6·0–9·5 mg/injection). The details of the various extraction procedures except those for alcohol extracts have been given previously (Howie, 1961). Alcohol extracts were prepared by covering the precipitate after centrifugation with alcohol and allowing extraction to proceed for four days with occasional shaking. The solvent was then decanted, filtered and evaporated to yield a yellow oily residue. Alternatively, the emulsion after centrifugation was dried in an oven at 40–60° C. The dry residue was then ground up and extracted by alcohol in the manner just described. All the extracts used were known to be potent in inducing the spawning of male worms. Controls were given either 0·5 M saline or arachis oil depending on which of these substances was used as a carrier for the extract in the experiment concerned. Worms to be injected were placed in 1 l. crystallizing basins filled with sea water. It was found best to keep the worms in the laboratory overnight before making the injections to permit evacuation of the gut as the presence of sand in the dishes made it difficult to detect the presence of eggs if these were shed in small quantities. Injections were made into the body cavity through the ventral body wall—care being taken to avoid the nerve cord and the blood vessels. The importance of maturation in shedding has already been demonstrated. Thus, whether shedding occurred or not, artificial fertilizations of coelomic eggs were usually attempted 5–7 h after the injection of extract, it being argued that any indication of the onset of maturation would suggest the presence of a substance in the extract influencing spawning. If eggs were shed an attempt was made to fertilize these to check the normality of the shedding reaction. As in males, injection of crude extract produced violent contraction of the body wall often resulting in splitting of the tail region with emission of coelomic eggs. Such contraction was rare

when fat solvent extracts were injected, nevertheless the presence of eggs in the dishes in which the worms were contained did not necessarily indicate that shedding had occurred. It was necessary therefore to maintain a constant watch on the worms in the period immediately following the injection of extract. The results of these experiments are summarized in Table 3.

TABLE 3. EFFECT OF INJECTING TISSUE EXTRACTS INTO FEMALE WORMS

Extract administered	No. injected	No. 'puffing'	No. spawning	Average time after injection (mins)	
A. Assays run for 24 h					
Crude	18 (12)	1 (1)	0 (0)	—	
Alcohol	28 (22)	10 (10)	4 (4)	162	
Ether	52 (12)	8 (5)	12 (7)	81	
Control	23	0	0	—	
B. Assays run for more than 24 h during the breeding season					
				No. of spawners in < 3 days	> 3 days
Benzene	36	2	10	7	3
Control	21	—	4	1	3

Figures in parentheses indicate numbers of worms in experiments conducted prior to the breeding season.

Experiments prior to the breeding season

In experiments carried out up to 3 weeks before the beginning of the breeding season it was common to find that at about 45 min. after administration of extract small puffs of whitish material were ejected from the nephridiopores. Thereafter 'puffing' continued intermittently for up to 5 h. The ejected material proved to consist of concretions of coelomic cells (not eggs). About half the worms given alcohol and ether extracts but only one worm given crude extract reacted in this way (Table 3). Four out of twenty-two worms given alcohol extract and seven out of twelve worms given ether extract emitted a few eggs at intervals varying between 30 min. and 4 h after injection—usually preceded by puffing. In no case were sufficient eggs shed to form a discrete egg puddle and they proved to be unfertilizable. No control worms spawned. It was thought that the 'puffing' reaction could only be interpreted as indicating some stimulation of the spawning mechanism and that the paucity of shedding was due to the fact that the eggs were insufficiently ripe.

Experiments during the breeding season

In the first instance mainly ether extracts were used as these appeared to be the most potent in inducing shedding. There was however a decreased response to injection of extract in these experiments. Of the forty worms given ether extract only three showed the 'puffing reaction' and five shed eggs while none of the six worms given alcohol extracts 'puffed' or spawned

(Table 3). Again eggs were shed in small numbers and only one sample proved fertilizable. In this, the embryos developed to ciliated stages but failed to hatch. Artificial fertilizations were attempted with coelomic eggs from all six worms given alcohol extract and twenty-eight worms given ether extract. Development occurred in six samples, none of which came from worms which had spawned. In four of these samples all the embryos died without hatching, but in two a few larvae hatched and survived for 11 days. There was no development when fertilizations were attempted with samples of coelomic eggs from seven control worms. The extracts appeared to be inducing some degree of ripening in coelomic eggs (cf. results of fertilizations with coelomic eggs from spent worms, above) and an improvement seemed possible if the extracts were allowed to act for a more prolonged period. Unfortunately, when the experiments were run for more than 24 h, the frequency of spawning among control worms (one in five) was almost as great as in worms given fat solvent extract. Ten out of thirty-six worms spawned after injection of extract (Table 3B). Spawned eggs and coelomic eggs from five of these spawners were fertilizable producing hatches of larvae. It would seem likely that these worms were going to spawn naturally as similar results were obtained when fertilizations were carried out with eggs from all four control worms which spawned. The remaining five worms which spawned subsequent to administration of extract shed unripe eggs; coelomic eggs from these worms were also unfertilizable. As eggs spawned naturally are readily fertilizable it may be that these five worms were induced to spawn by the action of extract.

DISCUSSION

The course of this investigation has been marked by a number of contradictory results, perhaps the most striking of which occurred in attempts to fertilize coelomic eggs. At first, in common with reports in the literature, these were unsuccessful, even with eggs from worms collected during the short breeding season. Subsequently it was found that eggs taken from the body cavities of worms which had started to spawn could be fertilized and that normal development would ensue. Thus maturation begins in the body cavity, a fact that was confirmed by histological examination of eggs from partially spawned worms. This is in contradiction of Pirlot's (1933) view that eggs cannot be fertilized until shed but not of his actual observation, i.e. that eggs undergoing maturation can be found in the nephromixia. At this stage it was concluded that maturation only begins after the onset of shedding. However, in further experiments, coelomic eggs were taken from worms kept in the laboratory until the end of the breeding season and it was found that these could be fertilized and develop normally. Thus, not only does maturation begin in the body cavity but it *precedes* the onset of shedding. However, as two of the three worms from which the successfully fertilized eggs were

taken spawned within 12 h after the fertilizations were made it would appear that shedding follows immediately upon the onset of maturation. In this respect it is significant that when coelomic eggs from a partially spent worm were fixed simultaneously with eggs from the nephromixia and newly spawned eggs, they all proved to be at the same stage of meiosis.

The close coincidence of maturation and shedding would appear to be insufficient explanation for the repeated failure of previous workers to obtain fertilizations of coelomic eggs and for the failure of similar experiments at the beginning of the present work. In all these experiments some worms should have been obtained which were about to spawn and whose eggs would have proved physiologically ripe. The explanation may be, simply, that female worms which are about to spawn are not obtained by the normal methods of collection, i.e. by digging between head hole and cast. A good deal of indirect evidence can be accumulated to suggest that this is so. The only physiologically ripe coelomic eggs, obtained in the present work, from worms which had not begun to spawn, came from worms which had been kept in the laboratory (8–11 days) until the end of the breeding season. Further, in experiments in which tissue extracts were injected control worms did not spawn until they had been kept in the laboratory for a number of days. In the course of this work, conducted over a number of seasons, the author never recalls female worms spawning immediately after they were brought into the laboratory. The explanation for the failure to collect females which are about to spawn may lie in a change in their behaviour on the shore at this time. Ejection of spermatozoa from the burrows during spawning has been observed on numerous occasions (Newell, 1948; Howie, 1959; Duncan, 1960). In contrast the formation of an egg puddle at the surface has been recorded on only one occasion (Duncan, 1960). Preliminary experiments (unpublished) suggest that females of *Arenicola marina*, like those of *Arenicola claparedii* (Okuda, 1938), retain their eggs in the burrow after oviposition. To this end they may cease active casting or may dig deeper when they are about to spawn so that they are less likely to be collected. It is noticeable in Duncan's records (1960, Table 3, figs. 3 and 4) that when spawning occurs at the Isle of Man and elsewhere there is a decrease in the absolute numbers of females, whether mature or spent, in her collections, and that spent females are rare.

There seems little doubt that maturation of the eggs in the body cavity is the immediate cause of shedding. It has been shown that maturation immediately precedes spawning and that eggs undergoing maturation are shed automatically if they are injected into the body cavity of a worm which itself is not spawning. In the light of experiments with males (unpublished) in which the breakdown of sperm morulae to free spermatozoa in the coelom is sufficient to induce shedding, it would appear that in both males and females a change in the nature of the gametes associated with their final maturation is sufficient for them to be taken up by the nephromixia and shed. This may well be a

frequent occurrence in polychaets where the gametes develop in the coelom and are shed through coelomoducts or nephromixia. Reports of difficulty in bringing about fertilizations with gametes taken from the coelom are not uncommon, e.g. in *Arenicola cristata* (Child, 1900) and *Audouinia tentaculata* (Wilson, 1936), which might suggest that in these species maturation of the gametes coincides with shedding. Furthermore, Scott (1911) has shown that in *Amphitrite ornata* the nephridia will take up ripe eggs but reject those which are immature.

While final maturation of the gametes may be the primary cause of shedding in the lugworm it is not suggested that spawning is entirely passive. This was illustrated by the fact that when mature eggs were injected into non-spawning worms only about half the number of eggs injected could be recovered when shedding was completed. A similar result has been obtained when free spermatozoa were injected into the body cavities of non-spawning males (unpublished work). Although administration of tissue extracts failed to produce in females the free spawning induced in males the results appear to support the hypothesis that shedding is not entirely passive. In experiments which lasted for less than 24 h tissue extracts induced intermittent emission of concretions of coelomic cells from the nephromixia (puffing) in 20% of the worms injected and 17% shed a trickle of eggs. Control worms showed no reaction. When attempts were made to fertilize the spawned eggs they proved unripe in all but one case when only 17% of the eggs developed. In experiments run for more than 24 h it was noticeable that five worms given tissue extracts shed unfertilizable eggs. The fact that the worms can be induced to shed even a small number of immature eggs would appear to support the idea that spawning is not entirely passive and dependent upon the maturation of the gametes.

It would also seem safe to conclude, from the evidence of the 'puffing reaction' and occasional spawning, that there is an agent in tissue extracts which stimulates, at least to a degree, the active uptake of cells from the body cavity. Development occurred in six out of thirty-four fertilizations of coelomic eggs attempted less than 24 h after injection of extract. Eleven larvae hatched in two of these. Viewed in the light of results of fertilizations with coelomic eggs from non-spawning worms discussed above this might indicate the presence in extracts of an agent which also induces maturation. If this is so its effect is very slight. The fact that eggs shed in response to injection of extract were unfertilizable also indicates that the extracts largely failed to induce maturation. This probably explains the small number of eggs shed by the worms in these experiments. It would appear therefore that while release of lipid into the body cavity may have some influence on the spawning of female lugworm as has been suggested for males, the primary stimulus is one which induces maturation of the eggs. Work is proceeding on the possible influence of neurosecretion on maturation of the eggs.

SUMMARY

Experiments are described in which attempts were made to fertilize eggs of the lugworm taken from the coelom. These were unsuccessful except when the eggs were taken from spent or partially spent worms or from worms kept in the laboratory until they were about to spawn. It was concluded from this that maturation of the eggs precedes spawning but only by a matter of hours. That maturation begins in the body cavity was confirmed by histological examination of eggs from partially spent worms and criteria are given for the recognition of immature and mature eggs. Lack of success in previous attempts to fertilize coelomic eggs from non-spawning worms may be because females which are just about to spawn are not obtained by the normal methods of collection. It is suggested that this is due to a change in the behaviour of the worms associated with retention of eggs in the burrow after oviposition. With regard to the stimulation of spawning in female worms, it has been shown that maturation immediately precedes the onset of shedding. This, together with the fact that if eggs undergoing maturation are injected into a non-spawning worm they are shed automatically suggests that the primary stimulus for the onset of shedding is, simply, one which will induce maturation of the eggs.

Observations made during the above experiment together with the fact that worms can be induced to shed a few unripe eggs by injection of tissue extracts suggest, however, that spawning is not entirely passive and dependent on maturation. The lipid fraction of tissue extracts stimulates the active uptake of eggs from the coelom but it fails to induce maturation of the eggs and hence the free spawning produced in male worms. While the release of lipid into the body cavity may have some effect on the spawning of female worms (under natural conditions) similar to that previously suggested for males, it cannot provide the primary internal stimulus for spawning.

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THE EFFECT OF SALINITY UPON THE BORING ACTIVITY AND SURVIVAL OF *LIMNORIA* (ISOPODA)

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(Text-figs. 1–5)

Most of the available information about the effects of salinity upon the distribution of the marine wood-boring isopod *Limnoria* has been acquired through salinity measurements of water containing infected timber. As a result of these observations, the concept of a limiting salinity has arisen, i.e. the salinity below which *Limnoria* attack is so much reduced as to be negligible. The published field data suggest a limiting salinity between 15 and 16‰, although more extreme values are sometimes mentioned. 15‰ is the value given by Krabbe (1914) for Iceland, by Atwood & Johnson (1924) for the east coast of the U.S.A. and by Sømme (1940) for the Baltic. Miller (1926) gives an average value of 14·5‰ for San Pablo Bay, California. Kofoid & Miller (1927) quote the Danish Engineering Association's figure of 16‰. A value of 20‰ is given by van Groenou, Rischen & van Den Berge (1951) for Dutch waters, and by Chellis (1948) for the United States of America. A general figure of 16–20‰ is suggested by Calman (1936) without reference to locality.

Few experimental attempts have been made to detect the limiting salinity, and of these only those of Kofoid & Miller (1927) have been on a sufficiently large scale. These authors compared the amount of faeces deposited from *Limnoria* infected timber in water of differing salinities, for since the excavated wood is swallowed by the borer, the faecal deposit provides a suitable index of the boring activity. The quantities produced were estimated by eye and referred to a linear scale on which the amount produced in water at a salinity of 5‰ was taken as unity. This method was admittedly crude as no attempt was made to standardize the number of *Limnoria* used in each experiment, while the measurement of the faecal production was insensitive, since the unit quantity was itself very small. However, they concluded that 13‰ represented the limiting salinity.

Survival experiments to detect the limiting (not the lethal) salinity were also carried out by Kofoid & Miller (1927) who put the value between 12 and 16‰. Less reliable survival experiments are described by Henderson (1924) and White (1929) who give values of 14 and 19·5‰ respectively as the limiting salinity.

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The experimental method is more likely than the ecological to yield definite information about the effects of salinity upon the boring activity. Field studies suffer from the discontinuous distribution of the habitat, and the presence or absence of the borer may be due as much to the availability of timber as to the salinity of the water. Consequently, the present investigation has been made by means of a number of long-term laboratory experiments in which the boring activity has been measured at various salinities.

The cause of the reduced boring activity with lowered salinity could be ascribed to several factors. One possibility is that the extra energy required for osmoregulation is diverted from that available for boring activity. However, this hypothesis has been discounted by blood osmotic pressure determinations, which have shown that *Limnoria* has no control over the concentration of its body fluids.[1] In the absence of osmoregulation, it is likely that the reduced boring activity reflects a decline in tolerance to lowered salinities. This assumption has been tested during the present work by means of survival experiments. No recent work on the salinities lethal to *Limnoria* has been traced in the literature. Salinities reported to be lethal after 24–48 h are given as 4·7‰ (White, 1929), 11‰ (Henderson, 1924) and 6·5‰ by Kofoid & Miller (1927) who state that a value of 10‰ is 'ultimately lethal'. White (1929) investigated the effects on the survival of *Limnoria* of concentrated salinities prepared by adding sodium chloride to sea water. Lethal values for 12- and 15-day periods are given as 44·7 and 41·1‰ respectively. The experiments of White and Henderson were made on too few animals to be considered significant, and Kofoid & Miller's work ignored the number of *Limnoria* used since it was concerned with the deposit of faeces from naturally infested timber. The published evidence was of limited help, therefore, and further investigation was necessary.

MATERIAL

The *Limnoria* used were extracted by hand from naturally infected timber taken from Southampton Water where three species of *Limnoria* occur together in variable proportions. They are *Limnoria lignorum* (Rathké), *L. quadripunctata* Holthuis, and *L. tripunctata* Menzies. The mixed species were used in the activity experiments since it was not possible to maintain an adequate supply of all three species throughout the extended periods of the work. Separated species were used in the survival experiments.

The animals were kept in clean sea water for periods of at least 48 h prior to the experiments, to allow any damage resulting from the extraction technique to become apparent. Only active specimens were used.

The experimental salinities were made from distilled water and filtered Plymouth sea water. Approximate dilutions were made to the salinities required and the exact values determined by titration. The experiments were

[1] Eltringham, Ph.D. Thesis, Univ. of Southampton, 1957.

carried out in an unheated aquarium room. Daily readings of the water temperature were taken.

ACTIVITY EXPERIMENTS

METHODS

The apparatus used in these experiments consisted of a number of 400 ml. beakers containing sea water of various salinities. Each beaker contained a pair of deal test blocks measuring 45 × 45 × 10 mm with the grain running parallel to the long axis. The blocks were separated by a U-shaped piece of glass rod about 3 mm in diameter, and secured by an elastic band to a glass weight which ensured that the blocks were kept below the water surface. These blocks were soaked in water of the salinity under test before use. Alterations in the salinity through evaporation were reduced by covering each beaker with a sheet of polythene through which a fine pointed narrow-bore glass tube was passed for aeration purposes. A constant air supply, controlled by individual taps for each beaker, was bubbled through the water at a slow rate. Under these conditions the increase in the salinity of the water through evaporation was shown not to exceed 2% after 7 days.

The *Limnoria* used in the experiments were rinsed in water of the test salinity and dropped into the beakers from a pipette. Two different methods were used to evaluate the boring activity. In the first, the burrow lengths were measured to the nearest mm by means of a piece of piano wire graduated in mm. Grooves produced on the wood surface were included as well as burrows but were allotted an activity value half that of burrows of equal length. The second method of measuring the boring activity was used when it was desired to follow the progressive attack by *Limnoria* upon the test blocks. For this purpose, the volume of the faeces produced each week was taken as the index of the boring activity. The faeces were freed from exuviae and removed with a pipette to a graduated centrifuge tube and their volume measured after constant treatment in a hand-operated centrifuge.

Boring activity at reduced salinities

Two experiments were conducted, each for a period of 90 days, in which nine different salinities were tested simultaneously. Fifty *Limnoria* were used in the first experiment and 80 in the second. The water was changed regularly every few days, when all dead specimens were removed and replaced by an equal number of healthy animals. Thus there was a constant number of living *Limnoria* in the beakers during the course of the experiments and all variable factors were common to each beaker except that of salinity. A wide range of salinities was tested in Expt. I with values approximately to 35‰ (full-strength sea water), 30, 25, 20, 18, 16, 14, 10 and 5‰. From the results of this experiment, it was decided to examine a narrower range in Expt. II using salinities ranging from 8 to 22‰ at intervals of 2‰ with a control at

35‰. The mean water temperature in Expt. I was 17·6° C (range 15·5–19·9° C) and in Expt. II 14·9° C (range 10·2–18·5° C). In neither case was there a violent fluctuation in temperature.

Rate of attack

This experiment was continued for a period of 20 weeks. The experiment was designed to compare the rate of attack of *Limnoria* in full-strength sea water and at a reduced salinity (30‰). A hundred *Limnoria* were added to each of two beakers containing the experimental test blocks in water of the respective salinities. The water was changed weekly when dead specimens were removed and counted but not replaced.

RESULTS

Boring activity at reduced salinities

In order to allow for the different number of animals used in each experiment, the results of these experiments are given in Table 1 as the activity per 50 *Limnoria*. The boring activity declined sharply with the salinity but the activity at comparable salinities was less in Expt. II than in Expt. I. The most likely explanation for this difference is that the second experiment was carried

TABLE I. THE BORING ACTIVITY OF *LIMNORIA* AT REDUCED SALINITIES

Approx. salinity (‰)	Length of burrows and grooves (mm) per 50 *Limnoria*		No. of burrows per 50 *Limnoria*		No. of grooves per 50 *Limnoria*		Av. burrow length (mm)	
	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II
35	333	270	60	39	5	8	5·5	6·7
30	271	—	45	—	11	—	5·8	—
25	271	—	48	—	8	—	5·4	—
22	—	129	—	25	—	7	—	4·8
20	202	102	46	22	22	14	4·1	4·1
18	129	85	32	18	10	19	3·6	3·5
16	129	60	32	12	12	15	3·6	3·7
14	61	24	19	6	7	6	2·1	3·3
12	—	14	—	2	—	9	—	3·3
10	12	2	5	1	3	1	1·4	2·0
8	—	0	—	0	—	0	—	—
5	0	—	0	—	0	—	—	—

out at a colder time of the year and that the reduced temperature caused a reduction in the boring intensity. There is, however, little difference between the experiments in the average burrow length at comparable salinities. On the other hand, there is a considerable preponderance of burrows in Expt. I over Expt. II (194:98), but no appreciable difference between the number of grooves in each experiment, at equivalent salinities. These results suggest that the lower temperatures during Expt. II reduced the attack (number of burrows and grooves), but not the boring activity (average burrow length) once the animals had become established within the wood.

The lowered salinity caused a definite decline in the boring activity and no boring at all was registered below 10‰. However, it is not easy to deduce from the results the value at which the salinity becomes limiting. The choice of this value must be arbitrary, but may be made objective by considering the limiting salinity to be that at which the activity is 20% of the activity in full-strength sea water. Such an attack under field conditions would be negligible and unlikely to lead to the establishment of a permanent population. The percentage attacks in Expts. I and II are plotted in Fig. 1. Regression lines have been included ($P < 0{\cdot}001$ in each case). The limiting salinity, taken at the 20% activity level, is 12‰ in Expt. I and 15‰ in Expt. II. The points of zero activity shown by the regression lines differ by about 3‰ also. This figure illustrates the point not brought out in the previous analysis, that lowered temperatures bring about an increase in the value of the limiting salinity as well as a reduction in the total activity. Reduced salinities, therefore, have the greatest effect in retarding the boring activity of *Limnoria* at low temperatures. Fig. 1 suggests that boring would have been possible at a salinity in which no attack was registered under the colder conditions of Expt. II.

Similar values for the limiting salinities are obtained when the activity is examined from the standpoint of number of bores. 20% of the number formed at 35‰ is 12 in Expt. I and about 8 in Expt. II. The first figure falls halfway between the totals found at 10 and 14‰ (i.e. 12‰), while the second lies between the values recorded at 14 and 16‰ respectively.

Rate of attack

The results of this experiment are shown in Fig. 2 which includes the weekly volume of faeces produced as well as the progressive volume over the whole period of the experiment. The temperature data are the weekly averages of the daily water temperature readings. In order to allow for the death rates, the results have been expressed as the volume of faeces produced per *Limnoria* over each 7-day period. For the purposes of this calculation, all of the deaths recorded at the weekly change of water were assumed to have taken place halfway through the period.

Fig. 2 shows that the initial activity was greater than that at any subsequent period, although the water temperature was at its lowest. The activity decreased from the second to the fifth week, after which it roughly followed the fluctuations in the water temperature. Although the attack was lower at 30‰ than in full-strength sea water, the times at which changes in the rate of attack occurred were the same. From the fifth week, the relationship of activity with time did not depart significantly from a straight line ($P < 0{\cdot}001$ in each case).

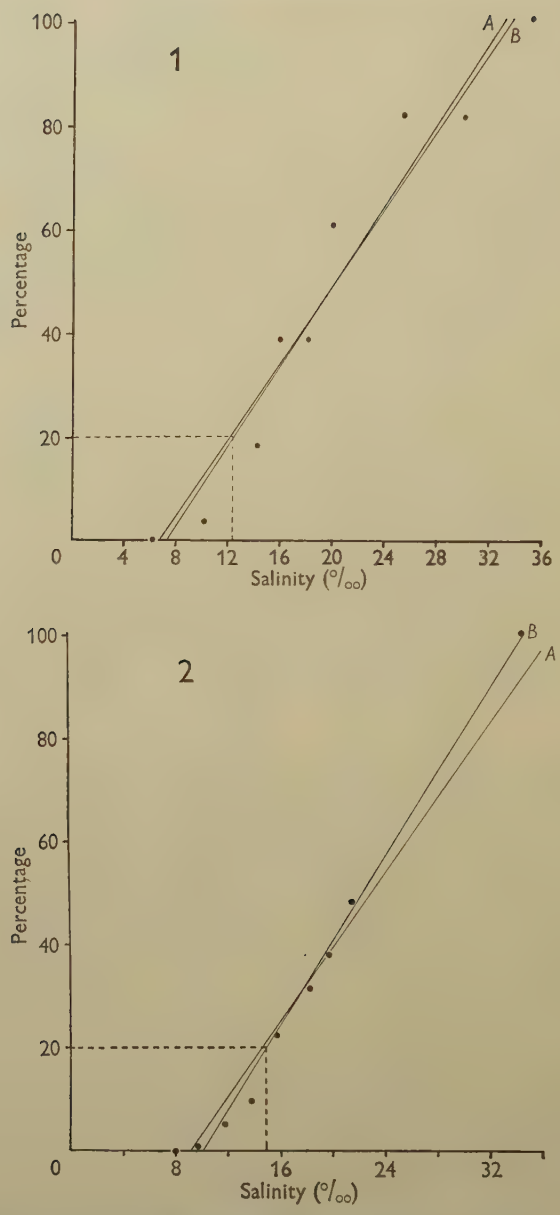

Fig. 1. The boring activity of *Limnoria* in relation to salinity: (1) Expt. I, (2) Expt. II. Each point shows the activity as a percentage of that in full-strength sea water (35 ‰). The dotted lines show the limiting salinity at the 20 % activity level. *A*, regression of *X* upon *Y*; *B*, regression of *Y* upon *X*.

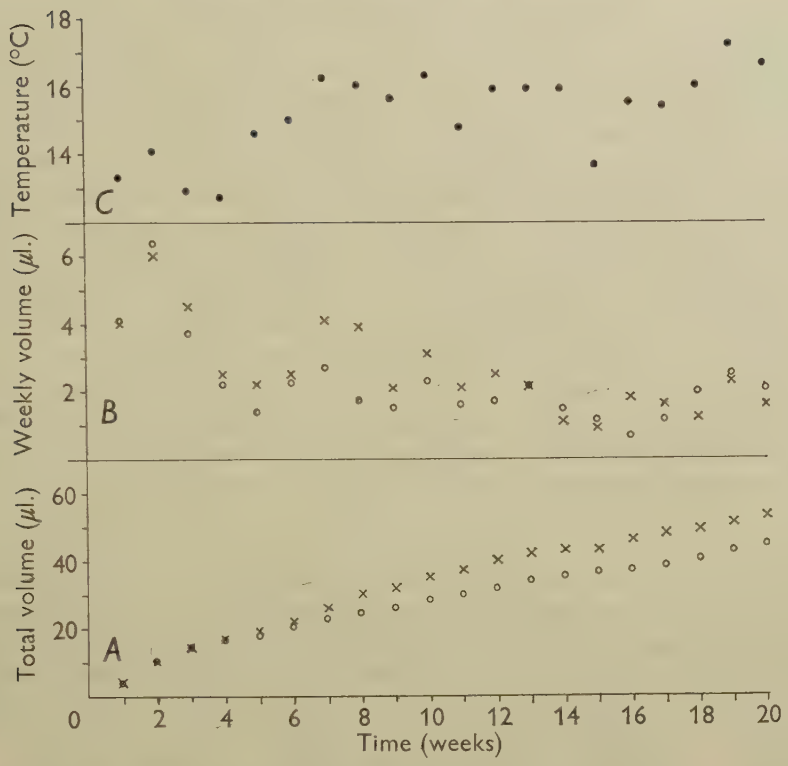

Fig. 2. The rate of attack on wood by *Limnoria*. *A*, The total volume of faeces produced per *Limnoria*; *B*, the weekly volume of faeces produced per *Limnoria*; *C*, water temperature. × faeces production at 36‰; ○, faeces production at 30‰.

SURVIVAL EXPERIMENTS

METHODS

The *Limnoria* were first washed in full-strength sea water, rinsed several times in the test solution and placed in 400 ml. beakers containing water of the experimental salinities. The top of each beaker was covered with polythene sheeting secured by a rubber band. In order to reduce evaporation, aeration was not provided, but the water was changed at least once a week. Each beaker was provided with two sheets of filter paper between which the *Limnoria* would crawl as this facilitated the counting of dead specimens at each inspection. Preliminary experiments showed that there was no significant difference in the survival between *Limnoria* with filter paper and those without. The criterion of death was complete immobility of all appendages when teased with a needle under a binocular microscope. Such individuals showed no sign of recovery when replaced in full-strength sea water.

The experiment, which compared the survival of *L. tripunctata* and

L. quadripunctata, extended over a period of 65 days. The survival of *L. tripunctata* was followed in water of eight different salinities approximating to 48, 42, 36 (full-strength sea water), 30, 24, 18, 12 and 6‰. The concentrated salinities were prepared by adding calculated quantities of Tidman's Sea Salt to Plymouth sea water. One hundred specimens of *L. tripunctata* were added to each of the eight beakers. A similar technique was used in measuring the survival of *L. quadripunctata* but, because fewer specimens were available, the number used in each beaker was 50, except in that containing 6‰ sea water, for which only 38 individuals remained. To make best use of the number of *L. quadripunctata* available, only six of the eight salinities tested with *L. tripunctata* were used; the values of 42 and 30‰ being omitted.

Both groups of beakers were inspected every day, when dead animals were counted and removed. The average water temperature during the entire experiment was 15·6° C; the lowest average weekly temperature recorded was 13·6° C, and the highest was 17·2° C.

RESULTS

Fig. 3 shows the survival at the end of 65 days. Each plot shows the number of *Limnoria* alive at a particular salinity by the end of the experiment as a percentage of the number alive in the control beaker containing full-strength sea water. In this way, allowance is made for the deaths in the control, for these

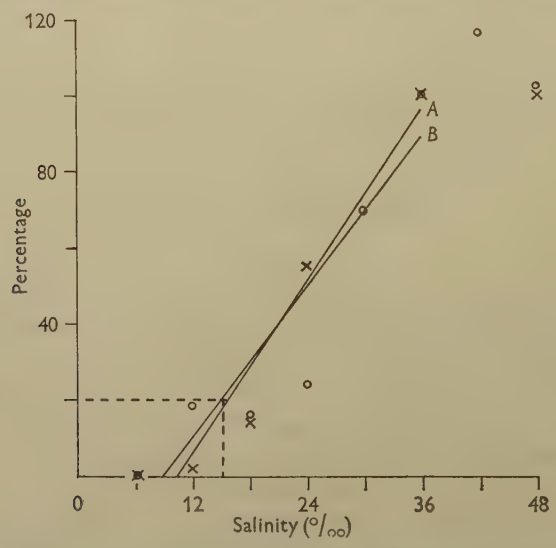

Fig. 3. The survival of *Limnoria* at various salinities. Each point shows the number of *Limnoria* alive after 65 days as a percentage of the number alive in full-strength sea water (36‰). ○, *L. tripunctata*; ×, *L. quadripunctata*. Regression lines have been drawn to the combined data excluding those for the concentrated salinities. The dotted line shows the limiting salinity at the 20% survival level. *A*, Regression of *X* upon *Y*; *B*, Regression of *Y* upon *X*.

presumably were not due to salinity effects. The number of deaths recorded in the control beakers was 26 (26 %) in the case of *L. tripunctata*, and 8 (16 %) for *L. quadripunctata*. A Student's *t* test conducted on the data for the two species (excluding the figures for the concentrated salinities) showed that there was no significant difference between the survivals of *L. tripunctata* and *L. quadripunctata*. Consequently, the data for these species have been combined in Fig. 3 in which regression lines have been included, again excluding the figures for the concentrated salinities. If the limiting salinity is taken as that at the 20 % survival level, similar to the method used in measuring the activity limiting salinity, the value is found to be about 15·5 ‰.

The rate at which the survival at reduced salinities decreased with time is given in Fig. 4, which shows the survival at the end of each 5-day period for the two species. Certain specific differences are seen from this analysis. The survival of *L. quadripunctata* was shown by a *t* test to be significantly higher than that of *L. tripunctata* in water of 24 ‰ salinity, but at 12 ‰ the survival was significantly higher in *L. tripunctata*. These results probably do not reflect a genuine specific difference, for it is unlikely that one species showing the higher survival at 12 ‰ would be less tolerant than the other of a salinity of 24 ‰. The survival of both species was declining rapidly at 24 ‰ and at this salinity range a considerable variation in the survival of the same species would not be unusual. The higher survival of *L. tripunctata* at 12 ‰, however, may indicate that this species is better able to withstand low salinities than *L. quadripunctata*. This is supported by the better survival of *L. tripunctata* in water of 6 ‰ which was the only tested salinity which proved lethal (i.e. caused 100 % deaths) within the period of the experiment.

There is no evidence to suggest that concentrated salinities up to a value of 48 ‰ have a deleterious effect upon the survival of *Limnoria*. The relatively high survival of *L. tripunctata* at 42 ‰ salinity may even indicate that increased salinity is beneficial up to a point.

DISCUSSION

It has been established that reduced salinity inhibits the boring activity of *Limnoria* and that its effects are the same on the initial attack (number of bores) as upon the boring activity within the wood (length of burrows). It has also been shown that the value of limiting salinity varies with temperature so that the concept of a limiting salinity range is more accurate than that of an absolute limiting salinity. The temperature effect is probably responsible for differences in the values of the limiting salinity reported by other workers since these were usually obtained from field observations under widely varying temperature conditions.

It is probable that there is a direct relationship between the boring activity and salinity although Kofoid & Miller (1927) drew a sigmoid curve on their

plots. A statistical analysis of the present results showed that the relationship was not significantly different from a linear one, and there is no evidence of high salinity range over which the boring activity was little affected.

The experiments on the rate of attack showed that boring was continuous and varied with the temperature. This evidence supports the belief that the primary function of boring in *Limnoria* is nutritional. The animal is known to ingest the wood, and Ray & Julian (1952) have shown that it possesses a cellulase and may therefore derive at least part of its energy requirements from the

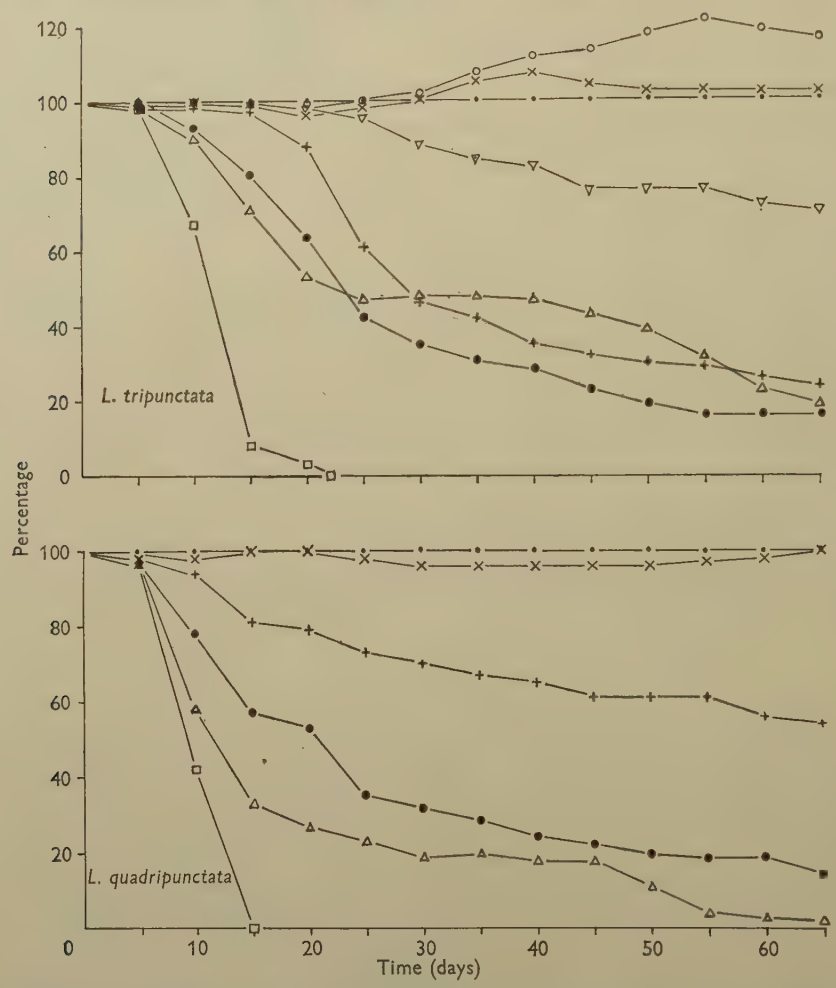

Fig. 4. The survival of *Limnoria* in waters of various salinities at the end of consecutive periods of 5 days' exposure. Each point shows the number alive as a percentage of that surviving in full-strength sea water (36 ‰). Key: ×, 48 ‰; ○, 42 ‰; •, 36 ‰; ▽, 30 ‰; +, 24 ‰; ●, 18 ‰; △, 12 ‰; □, 6 ‰.

wood itself. That an additional function of the boring habit is protective is suggested by the results of the experiment upon the rate of attack. The initial activity was maximal although the temperature was at its lowest. It is no doubt imperative at this stage that *Limnoria* should rapidly excavate a protective burrow before it is dislodged from the timber by wave action or destroyed by desiccation or predators.

The results of the survival experiments suggest that the reduced salinity had the same effect upon the boring activity as upon the survival. Comparison of the limiting salinities shows that the values of the survival- and activity-limiting salinities are similar at comparable temperatures (Table 2).

TABLE 2. COMPARISON OF LIMITING SALINITIES

Av. temperature (°C)	Type of limiting salinity	Value of limiting salinity (‰)
17·6	Activity	12·0
15·6	Survival	15·5
14·9	Activity	15·0

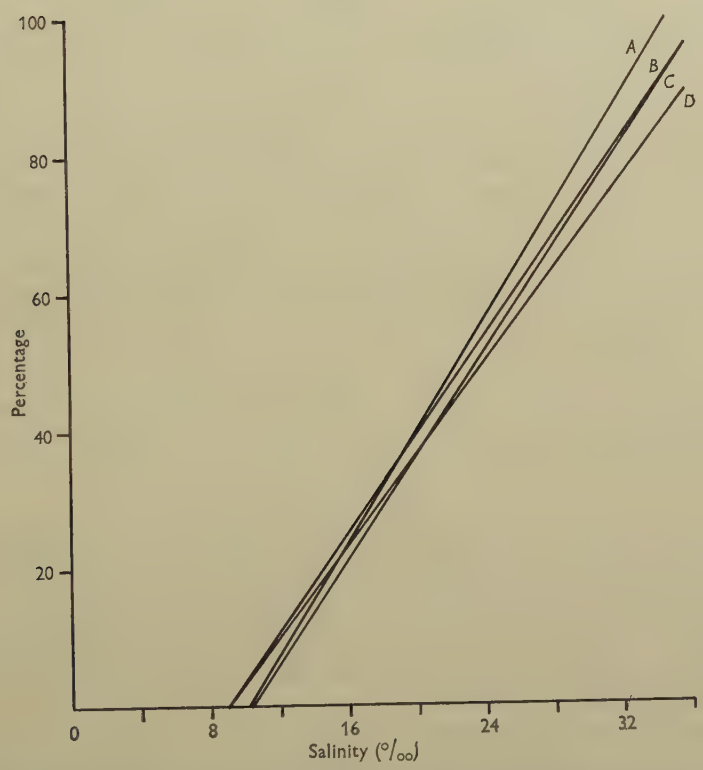

Fig. 5. A comparison between the effects of lowered salinity upon the boring activity and upon the survival. *A*/*B*, regression lines for the second activity experiment; *C*/*D*, regression lines for the survival experiment. The closeness of the lines suggests that the same factor affected the boring activity and the survival to the same extent.

A direct comparison between the effect of reduced salinity upon the boring activity and that upon the survival is given in Fig. 5. In this figure the regression lines calculated from the data for the second activity experiment and the survival experiment have been superimposed. These experiments have been selected as their average temperatures are comparable (14·9 and 15·6° C, respectively). The lines are close together indicating that the boring activity decreased with the lowered salinity in direct proportion to the lethal properties of the salinity. The comparison is not exact since the value of the survival limiting salinity varied with time as a result of differences in the death rate at the various salinities. However, these differences were considerable only during the first half of the experiment, and for the remainder of the time there were no large variations in the death rates. Consequently, the commensurate changes in the survival-limiting salinity during this period were slight. The value of the activity-limiting salinity is shown by Fig. 2 to be independent of time.

The survival experiments have important application in the field. The results suggest that temporary reductions in salinity through flood water, etc., can have little effect upon the survival and will not prevent *Limnoria* attack. The ability of *Limnoria* to tolerate concentrated salinities has an adaptive significance as the salinity of water within the burrows may rise appreciably when the timber is exposed at low tide on sunny days. However, the usual blanket of seaweeds and other sessile organisms would tend to reduce fluctuations in the salinity of the water within the burrows. The concentrated salinities (41–45‰) reported as lethal within 12–15 days by White (1929) had no effect upon the survival in the present experiment, and it appears that *Limnoria* is more resistant to low salinity than is suggested by other workers whose results are reviewed above.

I am greatly indebted to Mr A. R. Hockley for his guidance throughout the course of this work which was carried out during the tenure of a D.S.I.R. maintenance grant.

SUMMARY

The effect of salinity upon the wood-boring activity of *Limnoria* was investigated experimentally. Boring decreased linearly with salinity and ceased altogether below 10‰. The limiting salinity varied inversely with the temperature, being 12‰ at 17·6° C and 15‰ at 14·9° C (average temperatures). The boring activity was intensive in its early stages but after about 5 weeks fell to a lower constant rate.

Survival experiments suggested that the low salinity was the direct cause of the reduced boring activity. The only lethal salinity found during the period of the experiments (65 days) was 6‰ which proved fatal in about 15–20 days. The next higher salinity tested was 12‰. No reduction in survival was found in concentrated salinities up to a value of 48‰, the highest salinity examined.

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ON THE FAILURE OF THE PLYMOUTH HERRING FISHERY

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(Text-figs. 1–10)

In the winter of 1930–31 it was noticed that the winter maximum of phosphorus at the International Station E1 was less than the average of the previous 10 years. The change which has persisted ever since was associated with certain biological events (Kemp, 1938) of which the most important was the failure of the Plymouth herring fishery between 1931 and 1935, due to a failure of recruitment. Other changes included the replacement of *Sagitta elegans* by *S. setosa*; this change of a conspicuous and long-lived plankton indicator species was accompanied by changes of other indicators, implying a change of communities. At the same time a reduction was found in the quantity of macroplankton, as sampled by the wide-meshed ring-trawl. Certain species of medusae were no longer caught and the numbers of late summer medusae were reduced by four times (Russell, 1938*b*). A hint of a further change can be found in Russell (1939*b*) where he noticed that 'the western end of the Channel has been characterized by a great number of pilchard eggs and a general poverty of plankton indicators, including both species of *Sagitta*'.

From re-examination of the evidence available, including some new material hitherto unpublished, it is clear that a significant increase in pilchard occurred. The evidence for this increase is found in the regular ring-trawl catches taken off Plymouth, in marked contrast to the decline in the Plymouth herring fishery, which had no detectable effect on the numbers of young clupeids caught during the winter. The relative importance of the increase in pilchard leads to the hypothesis that the Plymouth herring fishery failed because the stock became reduced in competition with the pilchard population of the Western Channel, and that the change in the winter maximum of phosphorus was the effect of an increase in the number of pilchards.

I am grateful to Dr F. S. Russell, F.R.S., Director of the Marine Laboratory at Plymouth, for his permission to use some of Ford's unpublished data from 1932 to 1935, and to use other unpublished data on the occurrence of pilchard eggs in the Young Fish Trawl catches before 1935.

THE CHANGE IN ABUNDANCE OF THE POPULATION OF PILCHARDS

Once a week from 1924 to 1938, a ring-trawl was hauled for half an hour 2 miles east of Eddystone. In the results published (Russell, 1930, 1935, 1936, 1937, 1938*a*, 1939*a*), the clupeid larvae were not identified, but those caught in May, June and July may be classified as pilchards. Russell (1935) has written (p. 156): '. . .it is likely, therefore, that any clupeid occurring after the increase of sprat in February, March and April, will be pilchard right up to the end of the year when young herring begin to appear in December'.

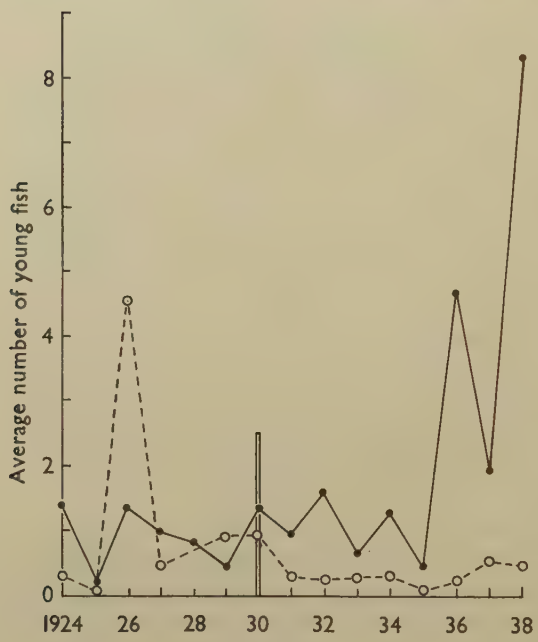

Fig. 1

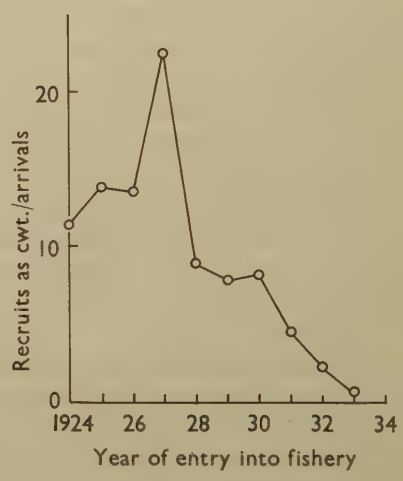

Fig. 2

Fig. 1. The numbers of young clupeids, assumed to be pilchards, caught off the Eddystone from May to July in 1924–38. The pecked line is the number of mackerel and horse mackerel ('scads').

Fig. 2. The numbers of recruit herring as at 3 years of age from 1924 to 1934.

Fig. 1 (from Cushing, 1957) shows the average number of young clupeids in May, June and July in each year for a long series from 1924 to 1938. Pilchard eggs were first observed in large numbers in the ring-trawl catches in the summer of 1935 (personal communication; Dr F. S. Russell); from Fig. 1, it will be noticed that the larvae first became abundant in 1936. At this time pilchards matured at between 3 and 5 years old; and from fat and maturity studies (Hickling, 1945), it is likely that a pilchard which ripened late in its

fourth summer spawned in its fifth (with four winter rings on its scales). The age-distributions given in Appendix II (p. 816) show that recruitment is spread over several age-groups. Fish spawned in the summer of 1930 may have matured by the summer of 1934 as three-ringers; later recruits would have appeared in 1935 and 1936. From the growth rate (see Appendix II), and from the pattern of recruitment, it is not likely that the full spawning potential was reached before 1936. Assuming that the outburst of eggs and larvae in 1936 really represents an increase in the adult stock, it is likely that the increased numbers of adults were born about 1930 or 1931. Dr Russell (personal communication) recorded pilchard eggs during the period 1930–35 as follows:

1930	1931	1932	1933	1934
652 (Nov.)	134 (Oct.)	present (Oct.)	Nil	Many pilchard (July and Sept.)

In 1935 he started to comment on the abundance of pilchard eggs, and in 1936 the number of pilchard eggs reached 5000 (Table 1). Thus the increase in pilchard eggs in *summer* agrees with the spreading of recruitment over perhaps three years. This suggests a more gradual increase in the stock than that shown by the larvae, which is a sharp increase in 1936; hence the number of pilchard eggs is probably a more precise index of stock, but there is a longer series of observations on pilchard larvae.

THE RELATIONSHIPS BETWEEN HERRING RECRUITMENT, NUMBERS OF PILCHARDS AND THE WINTER MAXIMUM OF PHOSPHORUS

Because the increase in pilchard stock appears to have started in 1930–31, it was decided to investigate its bearing on the other events that took place at that time. Three series of data were used: (1) on herring recruitment, (2) on numbers of pilchard eggs, and (3) on the maxima of winter phosphorus. They are set out as time series in Table 1. The values of herring recruitment (as calculated in Appendix I) are given as cwt./arrival at 3 years of age, but they are dated to the year of birth, i.e. as year-classes. The numbers of pilchard eggs are those observed in ring-trawl catches taken 2 miles east of Eddystone in May and June (Russell, 1930, 1935, 1936, 1937, 1938*a*, 1939*a*, 1947; Corbin, 1948, 1949, 1951 and personal communication; Southward, personal communication). The observations of phosphorus were taken at International Station E1 and are expressed in μg at. P/l. (Cooper, 1938; 1948, in which Station X3 is substituted for E1; Atkins, 1953, corrected for salt error; Armstrong, 1954, 1955, 1957, 1958).

Figs. 2 and 3 show the trend in herring recruitment from 1921 to 1930 (in year-classes) and the trend in winter phosphorus from 1921 to 1937. It appears that the decline in herring recruitment started long before the 1930 year-class,

possibly with the 1925 year-class and certainly with the 1928 one. The change in winter phosphorus is best interpreted as a declining trend. This conflicts with the view of Kemp (1938), who said that the change in 1930–1931 was a sudden one and suggested that the recruitment to the Plymouth herring fishery failed as a consequence. This latter interpretation must be considered unlikely because the major decline in herring recruitment started before 1930–1931.

TABLE 1. THE DATA ANALYSED

Year	Herring recruitment (cwt./arrival at age 3, dated to year of birth, December)	Pilchard eggs (monthly averages added for May and June; 2 miles E. of Eddystone)	Winter phosphorus (μg at. P/l. at E_1)
1921	11·4	No observations	0·65 (1921–22)
1922	13·8	No observations	0·71
1923	13·5	No observations	0·71
1924	22·5	(Present at E_1, Apr.)*	0·61
1925	8·8	(Present at E_2, July)*	0·76
1926	7·7	750 (2 miles west of Eddystone)†	0·55
1927	8·0	(Present in July)†	0·69
1928	4·4	?	0·69
1929	2·2	?	—
1930	0·6	—	0·52
1931	—	—	0·47
1932	—	—	0·53
1933	—	—	0·48
1934	—	—	0·42
1935	—	—	0·47
1936	—	5,000	0·48
1937	—	6,800	0·47
1938	—	6,100	—
1946	—	13,605	0·40
1947	—	12,226	0·45
1948	—	3,338	0·43
1949	—	781	0·51
1950	—	2,155	0·51
1951	—	2,490	0·46
1952	—	10,946	0·45
1953	—	4,962	0·51
1954	—	No counts	0·59
1955	—	1,812	0·50
1956	—	No counts	0·47
1957	—	8,650	0·44
1958	—	No counts	0·54

* Russell (1926). † Russell (personal communication).

It might be thought possible that the decline in herring recruitment was associated with the decline in winter phosphorus; as waters became less potentially fertile, the chances of survival in the ensuing summer could well decrease. The number of herring recruits has been plotted against the value of winter phosphorus in the winter of hatching; the regression (Fig. 4) is not significantly different from zero ($P = 0{\cdot}53$; $n = 9$) and it comprises the

evidence for the period 1921–1930. When the numbers of herring recruits are plotted against the value of phosphorus 1 year after the winter of hatching (see Fig. 5, which actually shows the plot made the other way), a surprising result emerges; the regression was highly significant ($P = 0{\cdot}01$; $n = 9$).

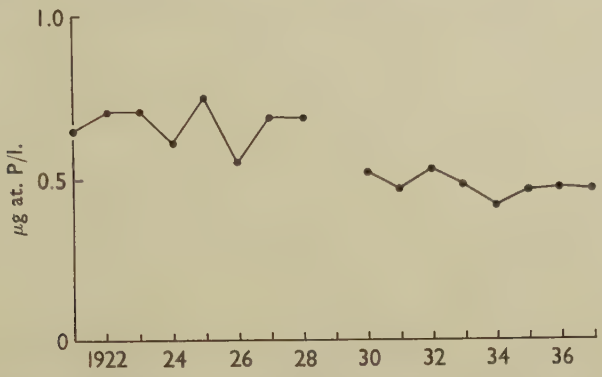

Fig. 3. Winter phosphorus at station E 1 from 1921 to 1937 (from Cooper, 1938). The year is that in which the winter began.

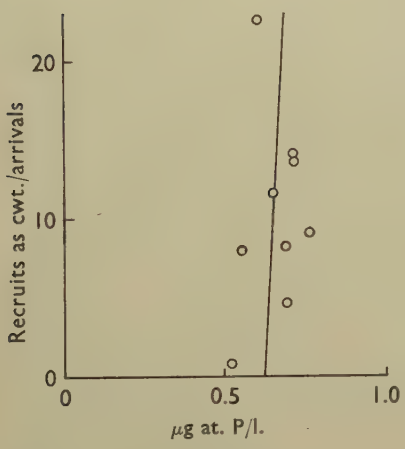

Fig. 4

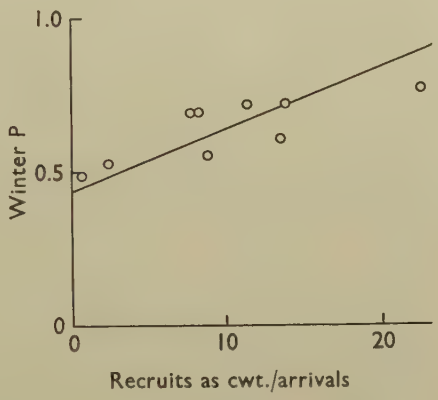

Fig. 5

Fig. 4. Regression of the number of herring recruits (3-year-olds) on winter phosphorus.

Fig. 5. Regression of winter phosphorus, 1 year after the herring recruits were hatched, on the number of recruits.

The evidence was compared for the same time period, 1921–1930. The contrast between the two regressions is striking, and it is the second one that requires explanation.

In general terms it appears that as the herring population declined in the early thirties, so the pilchard population increased, noticed as an increase in

eggs and larvae in 1935 and 1936. This is interpreted as an increase in adult stock, originating from eggs and larvae born about 1930 or 1931. No increase in eggs or larvae caught by the Young Fish Trawl occurred between 1930 and 1935, and so if the increase in the adult stock of pilchards were a true one, it must have sprung from an increased survival of larvae older than those generally caught by the Young Fish Trawl. It is of course possible that the adult pilchards came from somewhere else in 1935 and 1936. However, it is possible that the increased survival of pilchard larvae in 1930 and 1931 is associated with the striking regression of herring recruitment on winter phosphorus 1 year after the herring were hatched; the reason may be that pilchards spawn in May and June and the increased survival of their larvae from 1930 to 1931 could be interpreted as an end-point to competition between herring and pilchard, associated with the decline in herring recruitment which had been taking place.

Fig. 6 shows the regression of log numbers of pilchard eggs observed in May and June, and the value of phosphorus in the following winter (for the period 1936–1957, excluding 1954 and 1956). The regression is significantly different from zero ($P < 0{\cdot}01$; $n = 13$) and shows by extrapolation that if the numbers of pilchard eggs became reduced to low levels again (< 100), one would expect to find high values of phosphorus in the subsequent winter (0·65 μg at. P/l., which is close to the average value from 1921 to 1928, 0·66 μg at. P/l.). The observation of 750 eggs in 1926 with 0·55 μg at. P/l. in 1926–1927 has not been included in the regression because it is based on a single positive observation in May and June and four negative ones; if it is included the slope of the regression decreases slightly and the variance increases a little. Fig. 7 gives the regression of the numbers of pilchard eggs in May and June (log plot) on winter phosphorus, in the preceding winter, for the same period of years; this regression is not significantly different from zero ($P = 0{\cdot}2$–$0{\cdot}3$; $n = 12$). It will be seen that the two regressions given in Figs. 4 and 5 show a similar relation to each other as those shown in Figs. 6 and 7; in the first pair, numbers of herring recruits are relatable to winter phosphorus 1 year after hatching, and in the second pair pilchard eggs are relatable to the value of winter phosphorus 6 months after hatching. There is no relation between the numbers of herring recruits and the winter phosphorus in the year in which they were hatched, nor between that winter phosphorus value and the numbers of newly hatched pilchard eggs found 6 months later. The important point here is that both herring and pilchard indices are correlated with the same value of winter phosphorus, that of the following winter. There are two possible conclusions: (1) that both herring and pilchard are correlated with winter phosphorus; (2) that herring or pilchard is correlated with winter phosphorus, and that the other (pilchard or herring) is not correlated with winter phosphorus, but with the first.

It might be questioned here why the numbers of pilchard eggs have been used

in the analysis and not the numbers of pilchard larvae, for which there is more information. The reason is that the variability of regressions using pilchard larvae is very high and the distinction in the correlations given above does not appear. The larvae caught quantitatively by the Young Fish Trawl are not very old—about a month at the most—and so it is suggested that number of larvae is an index of stock, like the number of eggs, but not such a good one because of the effects of mortality and escape.

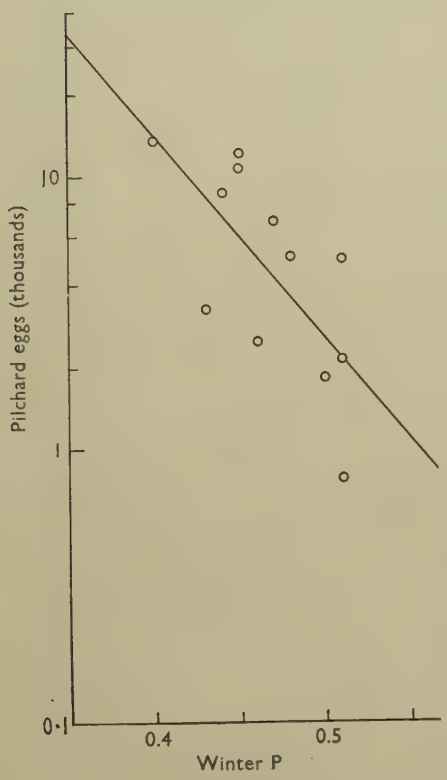

Fig. 6

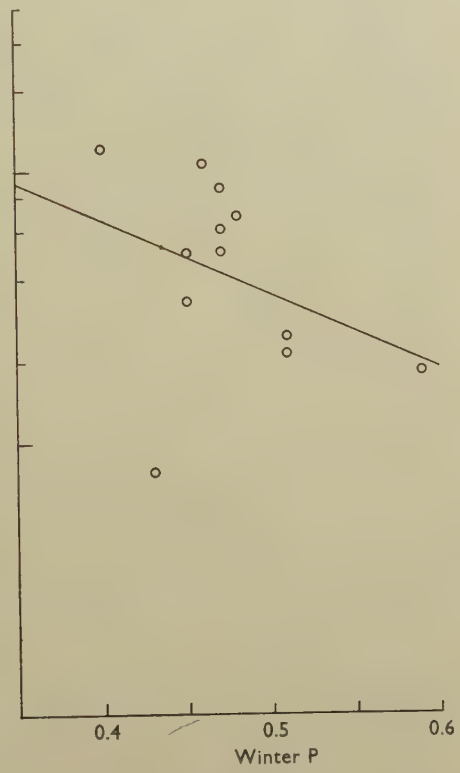

Fig. 7

Fig. 6. Regression of log numbers of pilchard eggs on phosphorus (in μg at. P/l.) in the subsequent winter. (Regression significantly different from zero: $P = 0{\cdot}02$–$0{\cdot}01$.)

Fig. 7. Regression of log numbers (thousands) of pilchard eggs on phosphorus (in μg at. P/l.) in the preceding winter. (Regression not significantly different from zero: $P = 0{\cdot}3$–$0{\cdot}2$.)

It seems likely that the low values of winter phosphorus from 1930–31 to 1935–36 were associated with the possibly increased numbers of pilchards of a size large enough to dodge the Young Fish Trawl. It might be argued that the herring/phosphorus relation and the pilchard/phosphorus relation were independent, the pilchards coming in from 1934 to 1936. This does not account

for the low values of phosphorus from 1930–31 to 1934–36, when herring recruitment was non-existent (except for 1930–31). It is, however, reasonable to associate the low values of phosphorus during that period with the possibly improved survival of older pilchard larvae (perhaps 6 months old—large enough to escape the Young Fish Trawl), which would also explain the stock increase in 1935–36. If it were conceded that the low values of phosphorus in 1930–35 were associated with higher numbers of 6-month-old pilchard larvae, the decline of herring recruitment in the earlier years might be accounted for in the same terms. In other words, there would have been competition between the pilchard and the herring, the herring recruitment declining from 1925 or 1928 onwards and the pilchard obtaining a decisive advantage in 1930 or 1931. Such an hypothesis would account for the regression of herring recruitment on the phosphorus values in the winters 1 year after the herring were hatched, because of the dependence of that winter phosphorus value on the number of pilchards hatched 6 months before. It is not a necessary part of this hypothesis that the significance of the winter phosphorus is known, because it is only a factor common to and connecting the two competitors. It is, however, tempting to suggest that the decline in the winter phosphorus represents an increment to the numbers of survived pilchard larvae—in other words, the lost phosphorus is locked up in living pilchard larvae of about 6 months old. If this were true then the stage of competition between the herring and pilchard populations would become a little clearer; herring in their first year competed with pilchards up to 6 months old. It is obvious in general terms that the pilchard replaced the herring off Plymouth in the early thirties; it is not noticed as an increase in catch because this is a minute proportion of the stock ($< 1\%$). It is suggested that the failure of recruitment to the Plymouth herring fishery was really a consequence of competition with the pilchards. We cannot distinguish between a failure of herring recruitment due to an independent cause (but continuous over many years), of which the pilchards took advantage, and a continuous and direct competition in which the pilchards were finally successful.

The numbers of pilchard eggs have been used as indices of adult stock. It has been suggested above that differences in the subsequent value of winter phosphorus are really differences in the quantities of 6-month-old larvae. We have enough data to relate these differences to that stock, when the 6-month-old larvae have recruited and have become one of the dominant age-groups in it. Fig. 8(A) gives the regression of winter phosphorus on pilchard eggs 4·5 years later, which is the time from 1930–31 to 1935–36; there is no relationship, the slope not being significantly different from zero ($P = 0{\cdot}9$; $n = 8$). In Fig. 8 (B) is shown a regression of winter phosphorus on pilchard eggs 4·5 years later for the pre-war period and 3·5 years later for the post-war period, taking into account recruitment at an earlier age in the post-war period; there is a slight improvement in the relationship ($P = 0{\cdot}2$;

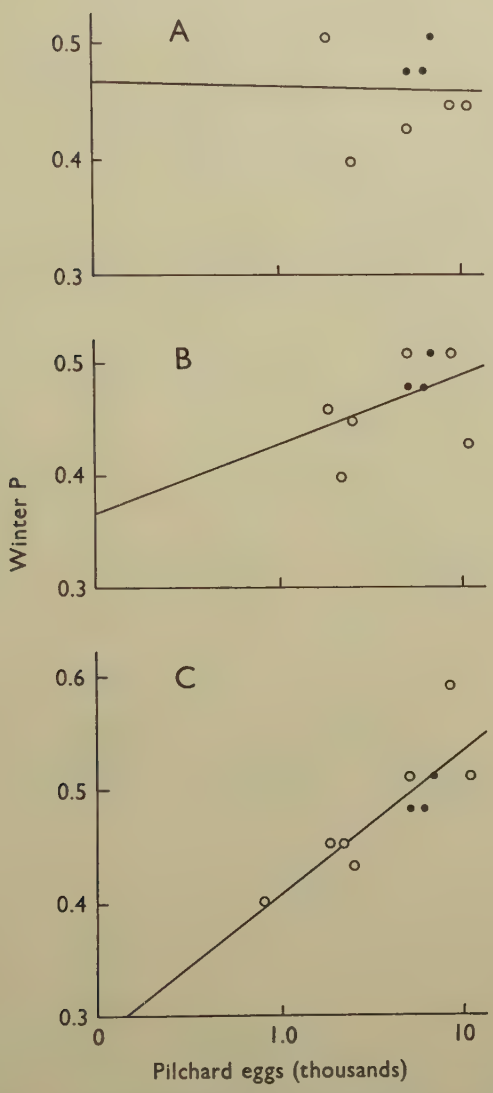

Fig. 8. A, Regression of winter phosphorus (in μg at. P/l.) on numbers of pilchard eggs 4·5 years later; pre-war observations are shown as black dots and post-war ones as open circles. (No significant difference from zero.) B, Regression of winter phosphorus on numbers of pilchard eggs 4·5 years later in the pre-war period (black dots) and 3·5 years later in the post-war period (open circles). (No significant difference from zero: $P = 0{\cdot}2$.) C, Regression of winter phosphorus on numbers of pilchard eggs 4·5 years later in the pre-war period (black dots) and 2·5 years later in the post-war period (open circles). (Significant difference from zero: $P = < 0{\cdot}01$.)

$n = 9$), but by itself of no significance. A third regression (Fig. 8 (c)) is of winter phosphorus on pilchard eggs 4·5 years later in the pre-war period and 2·5 years later in the post-war period ($P < 0{\cdot}01$; $n = 10$). This is a clear and good positive relationship which obviously depends upon the assumption that, during the post-war period, the fish have recruited earlier in their lives. There is independent evidence for this change (given in Appendix II). This relationship suggests that the differences in winter phosphorus really do represent differences in numbers of 6-month-old larvae.

We must now compare Fig. 6 (an inverse regression of pilchard eggs on phosphorus in the following winter) with Fig. 8 (c), the positive regression of winter phosphorus on pilchard eggs some years later. In the terms used so far, adult stock (pilchard eggs) is related directly to the 6-month-old larvae (winter phosphorus), which in its turn is inversely related to the adult stock some years later. It should be recalled that a low value of winter phosphorus is taken to indicate a large stock of 6-month-old larvae. Therefore adult stock was correlated (as pilchard eggs) 3 years later. Fig. 9 shows such a relationship, which is significantly different from zero ($P = 0{\cdot}03$; $n = 7$); when the stock is high, a low stock is to be expected 3 years later and vice versa. As a time series, the 3-year cycle cannot be seen decisively in the sequence of pilchard egg numbers, or in the phosphorus series, although some evidence of a cycle can be discerned. It will be noticed that only post-war data have been used and so the question of a possible change in the age of recruitment does not arise.

The pilchard eggs in the stock year are directly related to the 6-month-old larvae, as phosphorus, which are inversely related to pilchard eggs in the recruit year. The herring recruits were directly related to the phosphorus (in the year after they were hatched), that is, inversely related to the 6-month-old pilchard larvae. The latter appear to be directly related to the adult stock of pilchards, and so perhaps the mechanism of competition was a direct predation by adult pilchards on herring larvae. It is also possible that the cause of the 3-year cycle in pilchards is predation by adult pilchards on pilchard larvae. The more important point, however, is that a strict 3-year cycle should not occur when fish older than 3 years of age are found as a good proportion of the fishery. Thus, it is not surprising that the cycle appears neither in the time series of pilchard eggs, nor in that of phosphorus. Therefore, the correlation in Fig. 9 (from only six observations) suggests the presence of a cycle, but does not prove its existence, although there was a three-year gap in the observations.

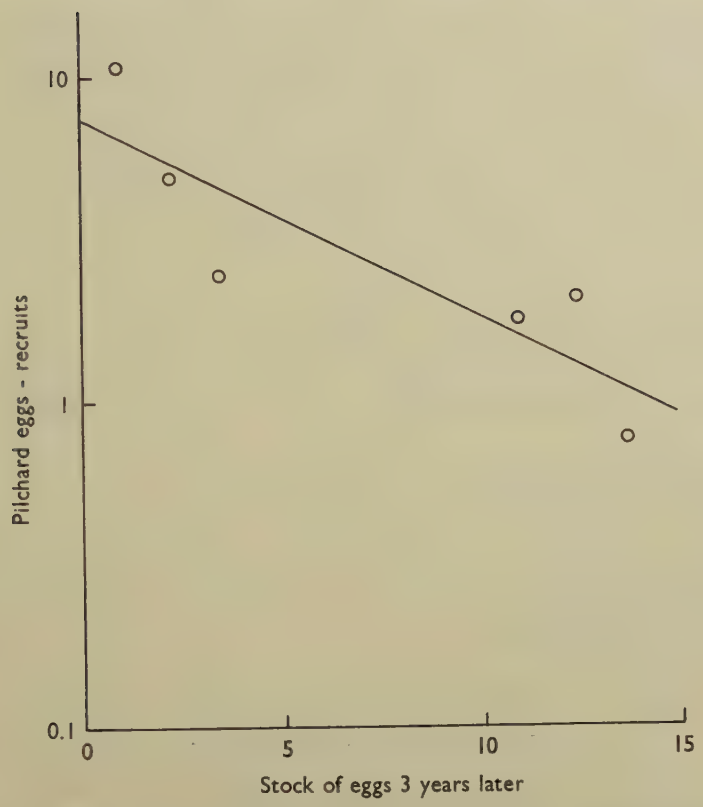

Fig. 9. Regression of log numbers (thousands) of pilchard eggs on pilchard eggs 3 years later. (For difference from zero: $P = 0{\cdot}03$.)

DISCUSSION

The arguments presented are given in summary and diagrammatic form in Fig. 10. The simplest interpretation of the relations is that differences in winter phosphorus are caused by differences in numbers of 6-month-old pilchard larvae; the development of the argument on stock and recruitment depends on this interpretation and reaches the point at which an independent and cyclic relation was discovered. This discovery strengthens the hypothesis that the decline of herring recruitment in the Plymouth herring fishery was associated with competition from pilchards. The association would have been quite decisive had there been independent evidence that the differences in winter phosphorus were truly associated with differences in 6-month-old pilchard larvae. This cannot be established and so the association must remain quite hypothetical.

Evidence of a relationship between stock and recruitment exists (Beverton

& Holt, 1957, give a convenient summary, with some analysis of the available data); but in general it would be true to say that the variability of such data prevents any relationship from appearing clearly. Beverton & Holt's analysis shows that if the relationship between stock and recruitment takes the form of a curve rising to a maximum of recruitment at a middle level of stock and falling to a low level of recruitment at high stock levels, then on the right-hand limb of such a curve, where recruitment declines at high stock levels, oscillations are inevitable. The fact that such oscillations have been detected in the

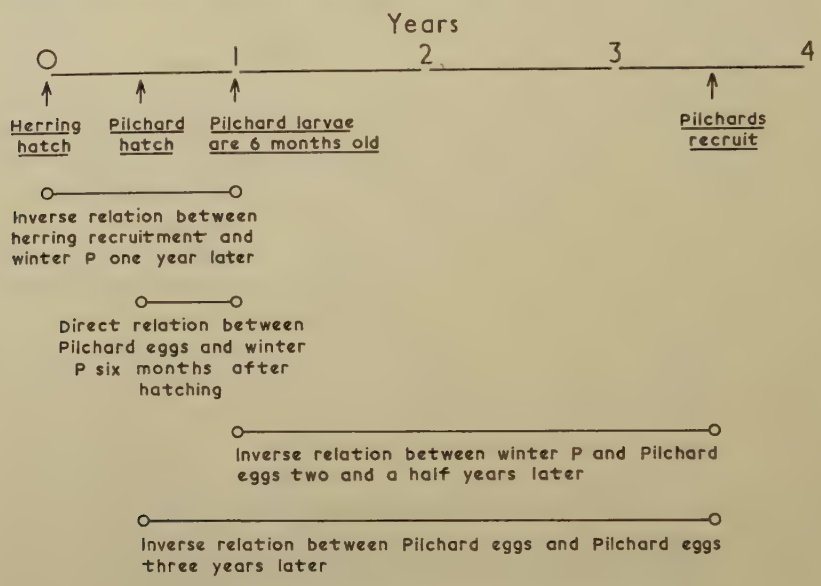

Fig. 10. The interrelationships of the regressions used.

case of the pilchard suggests that the stock and recruitment relationship is working on the high level of stock; indeed Fig. 9 can be regarded as part of the curve of recruitment on stock, showing the right-hand limb to the curve.

The cause of oscillation possibly is effective between the age of 6 months and recruitment. It was suggested that it might occur if adults ate larvae (Ricker, 1958); Beverton (1961) has suggested the term 'over-compensation' for such an effect in which above a certain level of abundance of present stock, the number of surviving progeny is reduced.

It was noted above that Kemp has interpreted the events of 1930–31 as an abrupt change and that the decline in winter phosphorus from 1924 to 1938 is here interpreted as a gradual change. It is likely that both are true in different senses. The change-over from rich to poor macroplankton, and from *S. elegans* to *S. setosa*, with its associated indicators, appeared to be abrupt. But Russell (1939) has pointed out that both species of *Sagitta* declined in

1936, which was the year of the pilchard increase—which is some evidence of gradual change. If, however, the decline in phosphorus is interpreted as a steady increase in 6-month-old pilchard larvae, this is a gradual change in competition with the herring, with a decisive step in 1930–31. The evidence that differences in phosphorus were associated with differences in numbers of 6-month-old larvae really rests on the relations with pilchard eggs both as parent stock and as subsequent recruits (Figs. 8(c) and 9). Therefore it is likely that the stock of 6-month-old larvae did increase in 1930–31 and that the changes in macroplankton and in plankton indicators came as a consequence. Thus the change in macroplankton might also have been a competitive change, the large and predatory jellyfish, etc., having been eliminated by the growing pilchards. A further consequence is that the plankton indicators have indicated not a change in water masses, but a change in communities as a result of competition.

There appears to have been a change-over from a community of herring, rich macroplankton, *S. elegans* and high winter phosphorus, to one of pilchards, poor macroplankton, *S. setosa* and low winter phosphorus. We know that there is an oscillation in the pilchard population and it is tempting to suggest that when the oscillation reaches a nadir, the quantity of 6-month-old pilchards is low and the winter phosphorus is high. In 1954–55 this happened, and in 1955, 1956 and 1957, young herring were caught in the Western Channel by trawlermen working from Brixham. It may well be that it is the pilchard oscillations that allow the herring population to obtain an advantage. In 1915 the winter phosphorus values were low (Matthews, 1917) and in 1917–18 the Plymouth herring fishery increased sharply in catch per unit effort. Is it possible that the remarkable point was not so much the decline of the Plymouth herring fishery in the early thirties, but its rise from 1918 onwards? It is possible that the present Dunmore fishery is subject to the same sort of effect, lying as it does on the edge of the pilchard distribution.

The winter maximum of phosphorus has been considered as an index of potential fertility. The argument presented in this paper does not necessarily deny this, but suggests that the proportion of living material in any phosphorus budget can be fairly high and so the winter maximum of phosphorus could only be at best a partial index of potential fertility. It has, however, been suggested elsewhere (Cushing, 1959*a*) that in certain tropical regions where phosphorus is very low all the year round the greater proportion of phosphorus is locked up in living material all the time. It was further suggested that this took place in a steady-state system and that the winter phosphorus was found in an unbalanced system of production, and that its magnitude was a rough and ready index of the backlash possible in such a system. If this were true it would imply that the real use of such measurements lay in the interpretation of changes in populations within different productive cycles, rather than in the measurement of potential fertility.

This paper does not seek to provide a factual account of the failure of the Plymouth herring fishery; this has already been done by Ford (1933). An hypothesis has been set up, postulating that the failure of recruitment to the herring fishery in the early thirties was an effect of competition with the pilchard population living in the same area. As such it is an alternative hypothesis to that put forward by Kemp (1938) to account for the biological changes that took place off Plymouth in 1930–31. The weaknesses of the present hypothesis are as follows.

(1) The information on pilchard larvae from 1924 to 1935 provides no evidence of stock changes or of competition. Particularly from 1931 to 1935, no increase in pilchard larvae took place. It is true that pilchard eggs were observed in 1934 and 1935, as might be expected from an increasing adult stock with partial recruitment. Further it has been suggested that numbers of pilchard eggs constitute a more precise measure of stock than numbers of pilchard larvae, and hence correlations with very low numbers of observations might be expected with eggs but not with larvae.

(2) The change of age of first recruitment is deduced from Table 3 in Appendix II for 1949–52; of these years, there is only one before the postulated change—1949. The change in length for age at the same time supports this conclusion, as does the treatment in Figs. 8(A–C). But the independent evidence of a change of recruitment would really require a *series* of age-distributions before 1950 and not one only.

(3) There is a lack of direct evidence from 1931 to 1935. It will be recalled that herring recruitment was correlated with the winter phosphorus one year after birth from 1920 to 1930, and pilchard eggs were correlated with winter phosphorus 6 months after birth from 1935 to 1958. It is possible that the two events are separate ones and that the low winter phosphorus from 1931 to 1935 was associated with a third independent factor. However, a regression has been established between winter phosphorus and pilchard eggs 2·5 years later, which means that the phosphorus must constitute a link between the pilchard stock and the pilchard recruits. It is reasonable then to assume that this link existed in 1930–35 and to name it 6-month-old larvae. On this point the whole hypothesis is simpler than that of three independent events. However, it is felt that even with these weaknesses there is sufficient coherence in it to justify serious consideration.

SUMMARY

An hypothesis has been erected to account for the failure of the Plymouth herring fishery and the associated biological events in the early thirties. It was found that the quantity of winter phosphorus was inversely related to the quantity of pilchard eggs 6 months earlier and directly to the quantity of herring recruits a year earlier. It is thought that the differences in quantities

of winter phosphorus have represented differences in numbers of 6-month-old pilchard larvae; the winter phosphorus provides the link between herring recruitment and numbers of pilchard eggs, suggesting that there has been competition between the herring and pilchard stocks, in their juvenile stages. Thus it is suggested that the Plymouth herring fishery failed in competition with the pilchard stock and that the other biological events which took place in the early thirties were a consequence of this postulated competitive change.

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APPENDIX I

The change in recruitment to the Plymouth herring fishery

The age-distributions sampled in the Plymouth herring fishery from 1924 to 1931 have been published by Ford (1933). The full age-distributions from 1924 to 1935 are given in Table 2.

TABLE 2. AGE-DISTRIBUTIONS SAMPLED IN THE PLYMOUTH HERRING FISHERY AS CWT./ARRIVAL OF EACH AGE-GROUP

Year	Age (years) 3	4	5	6
1924	2·2	4·7	22·3	6·3
1925	0·9	3·1	3·1	11·3
1926	0·7	4·0	3·6	1·9
1927	6·9	8·6	14·8	6·0
1928	0·7	7·6	5·3	7·6
1929	0·6	6·3	13·6	6·2
1930	1·1	3·1	7·5	10·9
1931	0·5	1·9	2·4	3·8
1932	0·9	2·6	4·6	4·0
1933	0·2	0·9	2·6	4·4
1934	0·3	0·1	2·2	2·0
1935	0·5	0·4	0·2	1·0

(*Note*. The published material, 1924–31, is of catches/unit effort based on returns from the merchants to Mr Ford. The unpublished material from 1932 to 1935, in percentages, has been raised by average figures of cwt./arrival based on the Sea Fisheries Statistical Tables; the bias introduced is considered to be very much less than the variation in abundance.)

If the age-distributions given in Table 2 are averaged, it becomes clear that the most abundant age-group is the 5-year-old, and so recruitment is assumed to have been complete by this age. To calculate the yearly number of recruits, some estimate of fishing mortality F, and of natural mortality M, is necessary. Some evidence has been presented that the instantaneous natural mortality rate of Downs herring in the North Sea amounted to 0·15–0·20 (Cushing, 1959*b*). The natural mortality of the Plymouth herring is, of course, unknown, but this value (which is close to that estimated for the Dunmore herring by my colleague A. C. Burd) can reasonably be used; if this value is an underestimate, its effect in the calculation is to reduce the magnitude of the estimates of recruitment. As we are only concerned really with relative values from year to year this possible bias does not matter very much. From the estimates of abundance given in Table 2 (1924–35), it is possible to make rough estimates of total instantaneous mortality rates from 5 to 6 years old (i.e. $\log_e N_5/N_6$ is an estimate of total mortality from the numbers/unit effort at 5 years old, N_5, and those at 6 years old, N_6; the average value of total mortality, $Z = (F+M)$, found was 0·45).

Let the total number of fish at 3, 4 and 5 years old be n_3, n_4 and n_5, respectively.

Let the number of recruits in each age-group, 3, 4 and 5, be r_3, r_4 and r_5; let the number of recruits appearing at 3, 4 and 5 years be r_3, $r_{3.1}$ and $r_{3.2}$ as at 3 years of age. We require $r_3+r_{3.1}+r_{3.2}$, the total number of recruits as at 3 years of age, or R_3. Let t be 1 year.

$$r_3 = n_3, \tag{1}$$

$$r_4 = n_4 - r_3 . e^{-Zt}, \tag{2}$$

$$\left.\begin{aligned} r_{3.1} &= r_4 . e^{Mt} = (n_4 - r_3 . e^{-Zt})\, e^{Mt}, \\ r_5 &= n_5 - r_4 . e^{-Zt} - r_3 . e^{-2Zt}, \\ r_{3.2} &= (n_5 - r_4 . e^{-Zt} - r_3 . e^{-2Zt})\, e^{2Mt}. \end{aligned}\right\} \tag{3}$$

So

$$\begin{aligned} R_3 &= r_3 + r_{3.1} + r_{3.2} \\ &= n_3 + (n_4 - r_3 . e^{-Zt})\, e^{Mt} + (n_5 - r_4 . e^{Zt} - r_3 . e^{-2Zt})\, e^{2Mt}. \end{aligned} \tag{4}$$

Fig. 2 shows R_3, the numbers of recruits as at 3 years of age from 1921 to 1930, the year being the year of birth of the year-class. There was a period of high recruitment from 1921 to 1924, after which it became reduced. Two periods of poor recruitment may be distinguished, 1925–28 and 1929–30; the latter period is one of very poor recruitment indeed.

APPENDIX II. RECENT CHANGES IN THE PILCHARD POPULATION

Since 1949, age- and length-distributions are available as sampled at Newlyn. The lengths for age are shown in Table 3.

As did the North Sea herring in 1950–52, the pilchard has increased in length for age, the 2-year-olds by 1·5 cm, the 3-year-olds by about 1 cm, and the 4-year-olds by about the same. Associated with the increase in length from 1950 to 1952, the two-ringed fish have become much more abundant, as shown in Table 4.

This change in recruitment is of the same character as that which took place in the

East Anglian fishery in 1950–51. However, it is not possible to distinguish between an earlier age of first recruitment and increased representation following a growth-change selection; there are no ring-net or trawl samples in the later years as in 1949. It is suggested from an examination of Table 4 that in 1949 the four-ringers were fully recruited and that after that date the three-ringers were fully recruited, and indeed a large proportion have come in a year earlier. The evidence is not complete, but it must be pointed out that the analogy with the change in recruitment to the East Anglian herring at the same time was found to be most suggestive.

TABLE 3. MEAN LENGTHS (CM) OF PILCHARDS IN THE COMMONER AGE-GROUPS, 1949–54

	Age (years)			
Year	2	3	4	5
1949	17·44	19·04	19·87	20·21
1950	17·71	19·82	19·92	20·66
1951	17·94	19·48	20·28	20·79
1952	18·90	19·81	21·62	22·46
1953	18·67	19·74	20·22	20·86
1954	18·95	20·23	20·80	21·15

TABLE 4. AGE-DISTRIBUTIONS OF PILCHARD IN THE WESTERN CHANNEL AS CWT./ARRIVAL

	Age (years)							
Year	2+	3+	4+	5+	6+	7+	8+	9+
1949	3	135	320	124	181	213	51	17
1950	57	253	194	156	217	240	30	14
1951	339	298	233	143	102	68	102	4
1952	144	182	147	158	171	112	68	41
1953	142	149	158	95	75	77	101	27
1954	85	134	229	149	144	45	—	—

Pilchards are spawned in midsummer and the l_1 (the projected length of the first summer zone on the scale) is comparatively large, 8–10 cm; this is rather like that of the East Anglian herring, which spawn in November and December and which are larger animals. Hence it is reasonable to assume that this value of l_1 represents more than a year's growth, perhaps 18 months'. In other words, it is unlikely that the first ring is laid down in the first winter of life, but in the second. For example, a fish hatched in the summer of 1954 would lay down its first ring in the winter of 1955–56 and its second in the winter of 1956–57. Consequently a two-ringed fish (2+) caught in the summer is about 3 years old.

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OBSERVATIONS ON THE BRITISH SPECIES OF *JAERA* (ISOPODA:ASELLOTA)

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(Text-figs. 1–4)

Two species of *Jaera* (Isopoda: Asellota) are recognized in Britain, namely *J. albifrons* Leach [= *J. marina* Bate & Westwood = *Oniscus marinus* Fabricius *nec* Linné] and *J. nordmanni* (Rathke) (see Bate & Westwood, 1868; Tattersall, 1906). Both species are found beneath stones and amongst algae in estuaries, in brackish pools and in or near freshwater streams running over the shore. In Britain the two have often been confused, particularly since Sars (1898) wrongly suggested that *J. nordmanni* described by Bate & Westwood (1868) might really be the male of *J. albifrons*. In addition, until very recently (Lemercier, 1960), little seems to have been written about the relative ecological distribution of the two species (see Kesselyak, 1938), though Stebbing (1876) suggested that near Torquay *J. nordmanni* occupied a higher zone on the beach than *J. albifrons*.

Though unrecorded in the revised edition of the *Plymouth Marine Fauna* (Marine Biological Association, 1957), *J. nordmanni* was found to be present in the district, as soon as it was looked for in the right places. Records by one of us (G. M. S.) are included in the Appendix (Table 2).

At present we aim to clarify some of the specific differences between *J. albifrons* and *J. nordmanni*, and also to compare the ecological distribution of the two species. General faunistic surveys of two restricted localities (McCartan & Slinn, 1953; Naylor & Slinn, 1958) have suggested that when the two species occur together, *J. nordmanni* extends farther into fresh water than *J. albifrons*. Present work on material from many localities in the British Isles confirms this view.

SUBSPECIATION

Forsman (1949) and Bocquet (1953) have recently made extensive studies on *J. albifrons* for which they have described five European subspecies (*albifrons*, *praehirsuta*, *forsmani*, *ischiosetosa*, *syei*). The subspecies are recognizable

in males only, on the basis of secondary sexual characters, and each is reported to have a characteristic habitat range. In Britain we have recognized all five subspecies, but observations suggest that the habitat preferences of the various subspecies in Britain are not so clearly defined as those described on the continent of Europe by Bocquet (1953, 1954), nor are they so clearly zoned according to tidal levels on the shore as is claimed in Europe. In addition, in Britain, the typical subspecies varies towards, and merges into, the condition of *syei*, particularly in the more brackish habitats. The separate identity of *syei* may therefore be in doubt, but the other four subspecies appear to retain their complete identity even when the populations mix. Intermediates, identifiable as hybrids from Bocquet's experiments, are quite rare.

Subspeciation has also been described in *J. nordmanni* by Lemercier (1960). Of the three subspecies which she has described, *J. nordmanni nordica* Lemercier is regarded as the only form found north of latitude 45° N., and so is likely to be the only one occurring in Britain. At present, though the subspecies of *J. albifrons* have been recognized, it is not possible to generalize about subspecific habitats. We propose, therefore, to deal mainly with the 'superspecies' *J. albifrons* and *J. nordmanni* and hope to discuss the subspecies problem in Britain at a later date.

IDENTIFICATION

The main characters separating *J. nordmanni* and *J. albifrons* are illustrated in Fig. 1. The body of *J. nordmanni* is more flattened and more widely oval in shape than that of *J. albifrons*. Furthermore, in the former species the sides of the head, thorax and abdomen are more setose, and the eyes are set more medially on the head. The male operculum, which lies ventrally, anterior to the pleopods, is narrow and pointed in *J. nordmanni*, whilst that of *J. albifrons* is T-shaped (Fig. 2).

A striking difference which has not apparently been reported, except in an unpublished thesis[1], concerns the size of adult males and females. When specimens are found in 'precopula' the male of *J. nordmanni* is almost invariably larger than the female, whereas in *J. albifrons* the male is smaller than the female. The mean lengths of ten randomly taken males and ovigerous females of each species from the Isle of Man and from Wales were as follows: *J. albifrons* ♂♂ 1·7 mm, ♀♀ 3·3 mm; *J. nordmanni* ♂♂ 3·3 mm, ♀♀ 2·8 mm.

The same feature was noted in all the South Devon collections. In the Broadsands sample of *J. nordmanni* taken on 3 May 1958 the five largest males were 4·6–4·2 mm body length (2·5–2·1 mm broad), while the largest females were only 3·7–3·2 mm long (1·8–1·6 mm broad), indicating an average length ratio (male to female) of about 5:4.

[1] On *Jaera marina* (Fabricius) and *Jaera nordmanni* (Rathke). G. Evans, M.Sc. Thesis, Univ. Coll., Aberystwyth.

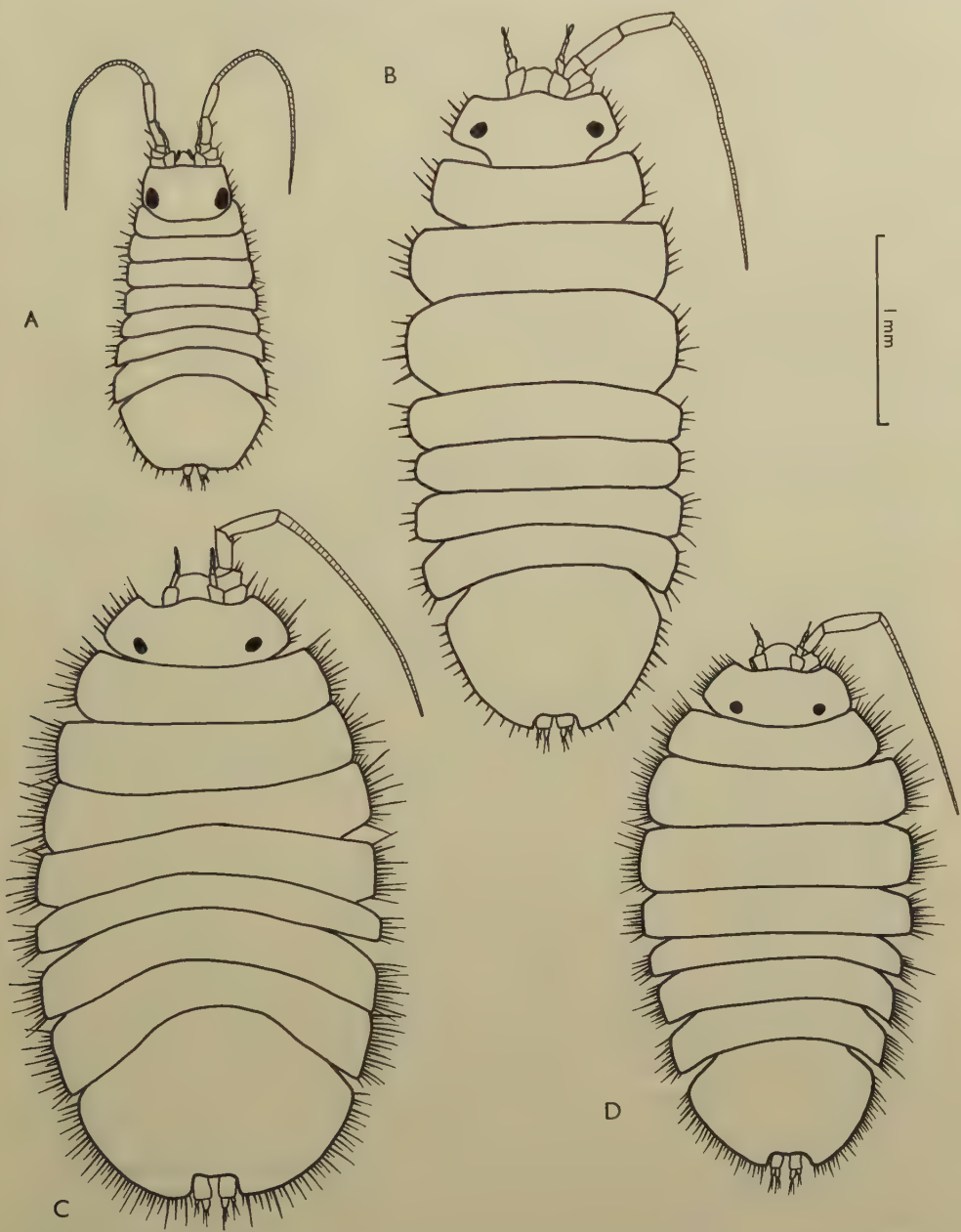

Fig. 1. A, *Jaera albifrons albifrons* ♂. B, *J. albifrons albifrons* ♀. C, *J. nordmanni nordica* ♂. D, *J. nordmanni nordica* ♀.

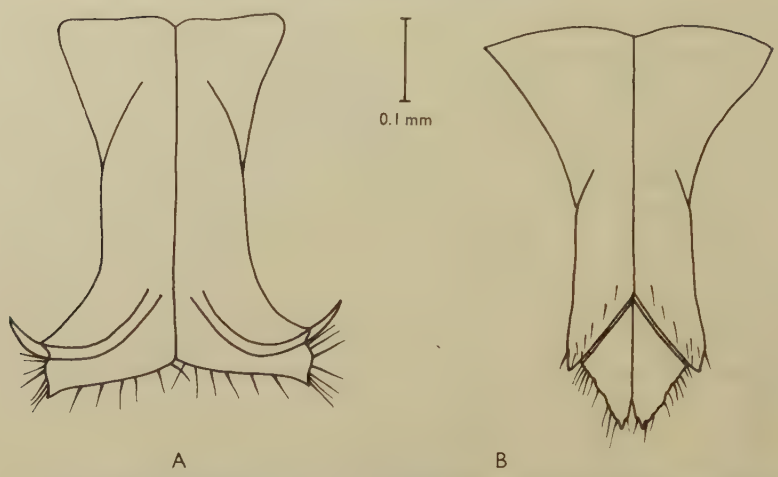

Fig. 2. Adult male operculum of (A) *Jaera albifrons ischiosetosa* (body length 1·5 mm) and (B) *J. nordmanni nordica* (body length 2·8 mm).

BREEDING AND SEX RATIOS

At Scarlett Point, Isle of Man, where periodical collections were made in 1954, ovigerous females of both *Jaera albifrons* and *J. nordmanni* were found whenever samples were taken, which was in January, February, April, June and November. It would seem therefore that some breeding took place throughout the year though, as was reported in Naylor & Slinn (1958), peak breeding for both species was in spring and summer. In the Yealm Estuary also, *J. nordmanni* was observed to breed at least from February to June in 1958.

In a total of 3556 *J. albifrons* collected from the Isle of Man, Wales and south-west England in the present investigations the ratio of males to females was 822:2734 or 1:3·3. Fewer *J. nordmanni* were sexed, but in a total of 676 the ratio of males to females was 347:329, which is approximately 1:1. The low proportion of male *J. albifrons* in the samples may be due to their having been partly overlooked owing to their small size (see p. 818), when the samples were taken. However, the numbers of males were consistently low even when particular care was taken to collect all the males and females from a sample, and the sex ratio seems to be primarily related to the method of sex-determination in that species. Staiger & Bocquet (1954, 1956) have demonstrated female heterogamety in *J. albifrons*; they have shown that there is a trivalent sex chromosome and also that a supernumerary chromosome may occur. The low proportion of males is apparently a direct result of these chromosomal complications. In *J. nordmanni*, on the other hand, the chromosomal complement seems to be constant at 24 pairs of bivalent chromosomes, and the species shows none of the chromosomal complications

observed in *J. albifrons* (Staiger & Bocquet, 1956). It thus seems likely that the method of sex-determination is normal in *J. nordmanni*, which would account for the approximate 1:1 ratio of males to females observed in the present investigations.

ECOLOGICAL DISTRIBUTION

Collections from under stones

At several localities in the British Isles where fresh water flows over the intertidal zone, specimens of *Jaera* were collected from under stones. Ten-minute collections were made at several tidal levels in each locality and the results of the more detailed of these surveys are given in Fig. 3. Tidal levels were determined with the use of surveying equipment in the harbours at Castletown and Ramsey, and also on the shores at Spaldrick and Poyll Vaaish, all in the Isle of Man. At Ramsey, Ordnance Datum was related to Chart Datum using the constant given in the *Admiralty Tide Tables* (1959). For the other localities in the Isle of Man the approximate data given by Southward (1953) have been used to make this conversion. In the streams on the shores at Llansantffraid, Ilfracombe, Newton Creek and Portwrinkle the tidal levels were roughly estimated from the water level on a calm day and from the zonation of algae. At all localities in Fig. 3 the relative positions of the tidal levels have been standardized for comparison.

Apart from the localities mentioned in Fig. 3 and Table 2 (p. 826) collections have been made in other parts of the Isle of Man, Wales and in the Plymouth area. Collections have also been examined from Cullercoats (Northumberland), Carnalea (Co. Down), Dublin, Jersey (Channel Islands) and Wrabness (Essex). All results confirm the general pattern illustrated in Fig. 3 in which it can be seen that *J. nordmanni* occurs from just below M.H.W.S. to M.T.L., usually being most abundant rather below M.H.W.N. *J. albifrons*, on the other hand, is not often found above M.H.W.N. and its numbers increase below that level as the numbers of *J. nordmanni* decline. Unlike *J. nordmanni* it is found down to about M.L.W.S.

Away from the influence of fresh water on the open shore the numbers of both species decline considerably. On the other hand, both species may extend relatively farther upstream on wave-beaten shores, depending upon the amount of sea water which is forced inland. At Scarlett Point in the Isle of Man both species occur in pools above M.H.W.S., with *J. albifrons* being restricted to the seaward end of the pools and *J. nordmanni* penetrating farther into fresh water (Naylor & Slinn, 1958). Owing to the indented nature of the coastline and the direction of the prevailing wind at Scarlett Point surf and spray periodically reach the pools. Similarly, on an indented coastline at Carnalea, Co. Down, *J. nordmanni* was found to extend some 2 ft. above M.H.W.S. (McCartan & Slinn, 1953). In an extreme case in the present investigation *J. nordmanni* was found beneath stones in a small stream on a

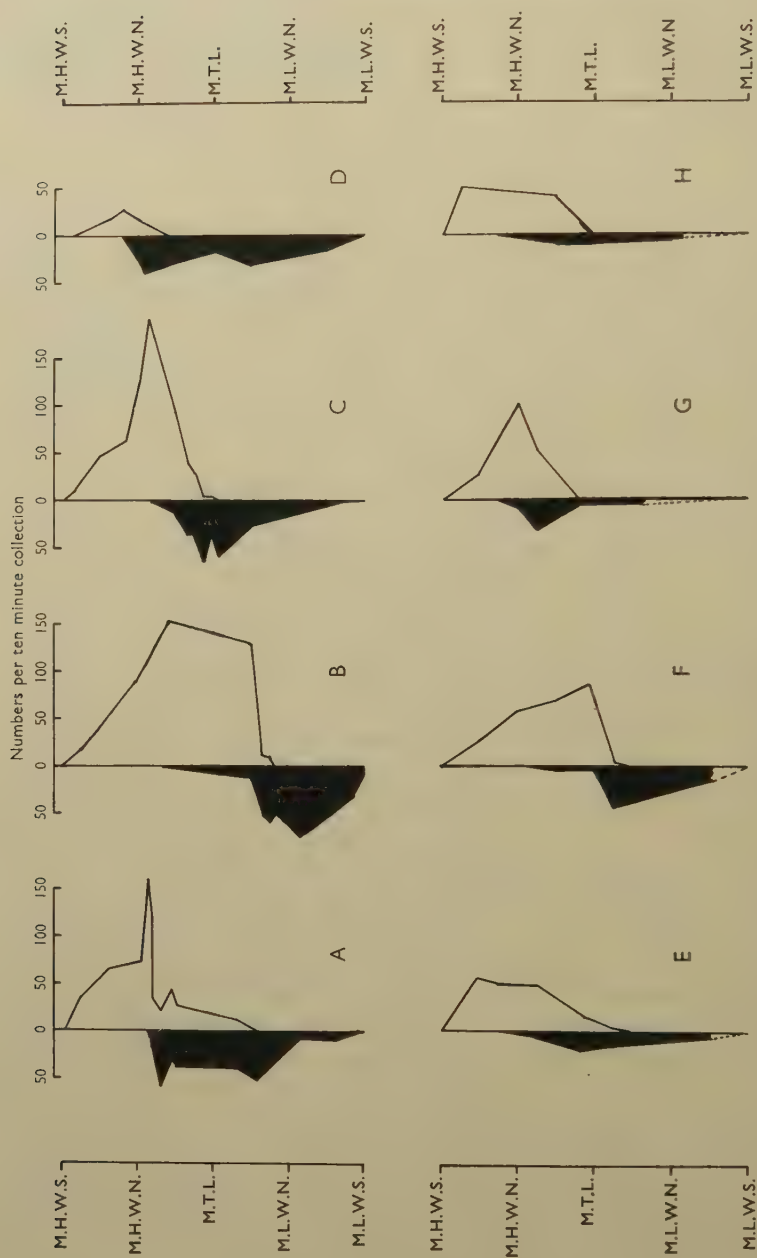

Fig. 3. Total numbers of *Jaera albifrons* and *J. nordmanni* in 10 min. collections beneath stones at various levels from M.L.W.S. to M.H.W.S. in sheltered estuaries at (A) Ramsey and (B) Castletown, Isle of Man, and in streams running over the shore at (C) Poyll Vaaish and (D) Spaldrick, Isle of Man, (E) Ilfracombe, north Devon, (F) Newton Ferrers Creek, south Devon, (G) Portwrinkle, east Cornwall, (H) Llansantffraidd, Cardiganshire. ●, *Jaera albifrons*; ○, *J. nordmanni*.

cliff face in the Calf of Man at a point approximately 150 ft. above sea level. The cliffs in that area face the prevailing south-westerly winds and spray would often reach the cliff tops.

Jaera was found to be most common under stones where the substrate was firm and consisted of gravel or muddy gravel. On rocky substrates, such as at Spaldrick (Fig. 3), *Jaera* was less abundant. In that locality stones were not embedded in the substrate and either provided insufficient shelter or were too unstable. Specimens were rare or absent from shingle banks and under stones in streams running over clean sand. In the last two types of habitat the substrate is presumably too unstable for populations of *Jaera* to become established.

Collections from amongst algae

Besides occurring beneath stones *Jaera* may often be found amongst algae which grow in or near streams which run over the shore and in estuaries. They are particularly abundant amongst algae in very sheltered estuaries but are much less common on algae than under stones on coasts exposed to only moderate wave action. On the moderately wave-beaten shore at Spaldrick (Fig. 3), a fairly sheltered part of Port Erin Bay, *Jaera* was virtually absent amongst algae. It was not found amongst *Fucus serratus* L. or *Fucus vesiculosus* L. and only single specimens could be found amongst *Ascophyllum nodosum* (L.) Le Jol and *Polysiphonia* sp. *Jaera* was more common amongst algae at Poyll Vaaish than at Spaldrick. Poyll Vaaish has about the same degree of exposure to wave action and wind as Spaldrick but the volume of fresh water flowing over the shore is considerably greater. Above M.L.W.N. at Poyll Vaaish small numbers of *J. albifrons* were found amongst *Fucus serratus*, *Gigartina stellata* (Stackh.) Batt and *Ascophyllum nodosum* at the edges of the stream. *Fucus ceranoides* L. in the stream contained considerable numbers of *J. albifrons* and, above M.T.L., a few specimens of *J. nordmanni*. Higher up, between M.T.L. and M.H.W.N., small numbers of *J. nordmanni* were found amongst *Enteromorpha* sp.

The two estuarine harbours investigated in the Isle of Man provided particularly sheltered conditions and *Jaera* was quite abundant amongst algae collected there. At Ramsey (see Fig. 4) *Fucus ceranoides* was examined from the level of M.L.W.N. to about M.H.W.N. where the weed became sparse and stunted. On this alga *J. albifrons* was common, being most abundant around M.T.L.; a few *J. nordmanni* were found between M.H.W.N. and M.T.L. At Castletown, *J. albifrons* was abundant on *Fucus ceranoides* from about M.T.L. almost up to M.H.W.N. In addition, at about M.H.W.N. the *F. ceranoides*, though sparse, contained fair numbers of *J. nordmanni*. In the Castletown estuary *J. albifrons* also occurred sparingly amongst *Enteromorpha* spp. between M.L.W.N. and M.H.W.N., and abundantly amongst *Ascophyllum* and *Polysiphonia* up to about M.H.W.N.

To compare the ecological distribution of the two species of *Jaera* where their ranges overlapped collections were made amongst *Fucus ceranoides* and under stones in the estuaries at Ramsey and Castletown, at a number of levels between M.L.W.N. and M.H.W.N. From these results it is not possible to make quantitative comparisons between the total numbers of *Jaera* under

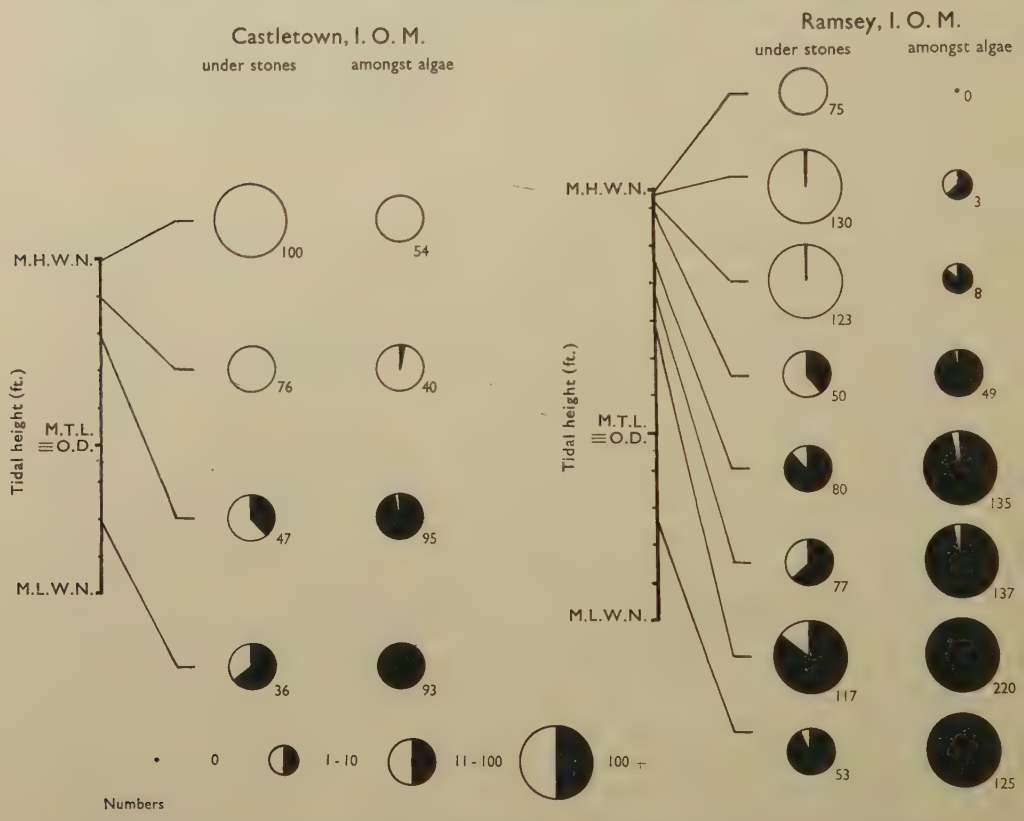

Fig 4. Relative distribution of *Jaera albifrons* and *J. nordmanni* under stones and amongst algae at various levels between M.L.W.N. and M.H.W.N. in estuaries at Castletown and Ramsey, Isle of Man. ●, *Jaera albifrons*; ○, *J. nordmanni*.

stones and amongst algae at any one level, but it is worth while to consider the relative numbers of each species in each type of habitat at the levels sampled (Fig. 4). At each level where both species occurred it is evident that the proportion of *J. albifrons* to *J. nordmanni* was consistently greater amongst algae than under stones. Conversely the proportion of *J. nordmanni* to *J. albifrons* was greater under stones than amongst algae. The significance of these results is discussed below (p. 826).

TOLERANCE OF REDUCED SALINITY

Preliminary work suggested that *J. nordmanni* was more tolerant of reduced salinities than *J. albifrons* (Naylor & Slinn, 1958) and those experiments have been confirmed and extended in the present work. In the present experiments specimens of both species of *Jaera* were kept in water of various salinities from 0 to 34‰, as far as possible under conditions of constant temperature, and the results of these experiments are combined in Table 1. It is clear that in water of salinity 3·4‰ and above, both species survived equally well for periods up to 4 days. *J. nordmanni* however, survived much better than *J. albifrons* in salinities below 3·4‰. In distilled water both species normally survived for only a few hours, but again *J. nordmanni* survived longer than *J. albifrons*.

TABLE 1. PERCENTAGE SURVIVAL OF *JAERA NORDMANNI* AND *J. ALBIFRONS* DURING SUCCESSIVE DAYS IN WATER OF VARIOUS SALINITIES

Total numbers alive at the start of each experiment in parentheses.

Time in days	*J. nordmanni*. Salinity (‰)				
	34	3·4	0·34	0·034	Distilled
1	100 (30)	96 (44)	80 (49)	68 (79)	6 (50)
2	100	91	82	66	—
3	100	86	65	53	—
4	100	82	47	50	—

Time in days	*J. albifrons*. Salinity (‰)				
	34	3·4	0·34	0·034	Distilled
1	100 (30)	95 (57)	58 (40)	49 (70)	3 (60)
2	95	88	33	37	—
3	95	87	27	—	—
4	95	78	17	—	—

DISCUSSION

It is clear that both British species of *Jaera* are euryhaline and are most commonly found where fresh water flows between tide-marks. The pattern of distribution, with *J. albifrons* occurring in the more marine part of the range and *J. nordmanni* penetrating farther into fresh water, persists throughout the year in localities which have been visited regularly (see also Naylor & Slinn, 1958). Experiments in which specimens were kept in water of reduced salinity show that *J. nordmanni* is more tolerant of very low salinities than *J. albifrons*. This clearly suggests that the former species might be physiologically better able to withstand the extreme variations in salinity which prevail in the more estuarine conditions where it is most abundant. Yet the results in Table 1 indicate that the two species tolerate equally well salinities of from 3·4‰ to 34‰. It would seem therefore that *J. nordmanni* is restricted to the more estuarine conditions by competition with the less euryhaline species *J. albifrons* (Naylor & Slinn, 1958).

Despite the fact that both species occur beneath stones and amongst algae it is evident that when the two species occur together in sheltered harbours the proportion of *J. albifrons* to *J. nordmanni* is greater amongst *Fucus ceranoides* and *Ascophyllum nodosum* than it is under stones at the same tidal level. On the other hand, *J. nordmanni* is relatively more abundant beneath stones, though admittedly algae are normally sparse at the upper limits of its distribution. This difference in habitat exhibited by the two species is not, however, generally applicable in a wider range of habitats. In slightly more wave-beaten situations, particularly where only a small volume of fresh water flows over the intertidal zone and where *Fucus ceranoides* is absent, then both species are rare amongst algae, even amongst *Ascophyllum* (p. 823). Also, in pools at Scarlett Point in the Isle of Man both species were found to be more abundant amongst algae than under stones when a choice of habitat was available (Naylor & Slinn, 1958). In that case it was possibly due to the *Jaera* seeking algae to avoid the more stagnant conditions beneath stones. When there is sufficient fresh water to immerse the algae and when the environment beneath stones is sufficiently well oxygenated, as is apparently so in the estuaries at Ramsey and Castletown, then mutual competition between the two species of *Jaera* is perhaps responsible for their ecological separation.

Such differences in the ecological distribution of the same species, and often of the same subspecies, made it difficult to generalize about subspecific habitats in the present investigation. As indicated earlier the habitat preferences of the subspecies of *J. albifrons* in Britain are not so clearly defined, nor so clearly zoned according to tidal level, as they are in Europe (p. 818). Finally, in Europe, *J. albifrons albifrons* Forsman is reported from beneath stones whilst *J. albifrons praehirsuta* Forsman is said to be particularly abundant amongst algae in the same localities (Forsman, 1949; Bocquet, 1953, 1954). In Britain, a similar separation of the two subspecies occurs near Plymouth. At Castletown, in the Isle of Man, however, both these subspecies occurred together amongst algae and under stones at several levels which were sampled. When more data are available it is hoped to discuss the subspecies problem in greater detail.

One of us (E.N.) is grateful to Dr F. S. Russell, F.R.S., for the provision of laboratory facilities at the Marine Laboratory, Plymouth, and to Mr J. S. Colman for facilities at the Marine Biological Station, Port Erin. Miss Winifred Chapman assisted with field work in the Isle of Man and Dr R. Howells kindly drew our attention to the unpublished M.Sc. Thesis of Miss G. Evans. Finally, we would like to thank the following people who have sent material: Dr H. O. Bull (Cullercoats), Miss M. Duhig (Dublin), Dr M. George (Dale Fort), Mr A. J. Hopson (Flatford Mill) and Mr R. F. le Sueur (Jersey).

SUMMARY

Brief descriptive notes, with comments on subspeciation, breeding and sex-ratios, are given for *Jaera albifrons* and *J. nordmanni* in Britain. The ecological distribution of each species is described. Both species occur amongst algae and under stones in estuaries, brackish pools and in freshwater streams running over the intertidal zone. *J. nordmanni* normally occurs from just below M.H.W.S. to about M.T.L., usually being most abundant just below M.H.W.N. *J. albifrons* is rarely found above M.H.W.N. and it extends down to about M.L.W.S. Experiments show that *J. nordmanni* survives better than *J. albifrons* in water of reduced salinity.

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APPENDIX

TABLE 2. *JAERA NORDMANNI* (RATHKE): OCCURRENCES IN THE PLYMOUTH AREA (G. M. S.)

Locality	Date	Tidal zone	Stones or weeds	*Jaera nordmanni* ♂	*Jaera nordmanni* ♀	*Jaera nordmanni* total	*Jaera albifrons* total	Fauna notes
Plymouth Sound: Jennycliff Bay (close to freshwater stream over shore)	4. iii. 58	Upper shore (*Fucus vesiculosus*)	S	4	0	4	31	*Procerodes ulvae*, *Protodrilus*, *Enchytraeus*, *Marinogammarus stoerensis*
		Middle shore (*Ascophyllum*)	S	1	0	1	111	*Marinogammarus stoerensis*
Heybrook Bay, opposite Renny Rocks (no stream, at most irregular freshwater seepage)	21. v. 58	H.W.N.	S	39	43	82	55	*Monodonta* common
Renny Rocks, rock pool (salinity 17·6 ‰)	21. v. 58	Pool in splash zone	S	11	5	16	0	Large *Procerodes ulvae*, *Gammarus duebeni*, *Ligia*
Yealm Estuary, south shore, freshwater stream over shore	22. iii. 58	H.W.S. (*Enteromorpha*)	S	12	12	24	0	*Gammarus duebeni* common, *G. zaddachi*
		H.W.N. to M.T.L.	S	36	26	62	34	*Procerodes ulvae*, *Marinogammarus stoerensis*
		Below M.T.L.	S	0	0	0	10	*Melita palmata*
Yealm Estuary, Cofflete Creek: Bridge at extreme head	25. ii. 58	River channel, running fresh	W	7	7	14	0	*Asellus aquaticus*, *Potamopyrgus jenkinsi*, *Gammarus zaddachi*
50 yards down from bridge, small freshwater trickle over shore	25. ii. 58 13. iii. 58	*ca.* H.W.N.	S	9	4	13	0	— — —
300 yards from bridge	25. ii. 58	Upper shore	S	4	3	7	115	—
300 yards from bridge	25. ii. 58	Upper shore (*Fucus ceranoides*)	W	0	0	0	0	— — —
Aune Estuary, station B4	8. vi. 58	Mid to upper shore	S	1	1	2	0	*Marinogammarus marinus*, *Gammarus salinus*, *G. zaddachi*
Aune Estuary, Station B6	8. vi. 58	River channel	W	2	0	2	0	*Gammarus zaddachi*
Torbay: Broadsands Marsh:								
Stream inside sea bank	3. v. 58	Stream channel	S + W	72	47	119	0	*Gammarus zaddachi*, *Melita pellucida*
Sandy shore where the stream flows over it	3. v. 58	M.T.L.	S	47	39	86	0	—
Sandy shore where the stream flows over it	3. v. 58	Below M.T.L.	S	0	0	0	0	—

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

ATKINS, D., 1961. A note on the growth stages and structure of the adult lophophore of the brachiopod *Terebratella* (*Waltonia*) *inconspicua* (G. B. Sowerby). *Proc. zool. Soc. Lond.*, Vol. 136, pp. 255–71.

Certain of the growth stages of the lophophore of *Terebratella* (*Waltonia*) *inconspicua* (Sowerby), from a shell length of 0·55 mm, are described and figured, so as to elucidate the position of the mouth and the mode of development of the lip of the food groove. The mouth is a small aperture in front of the bases of the first formed pair of filaments. The lip of the food groove is formed by lateral growth from a small preoral lobe. The early lophophore (trocholophous and early schizolophous) is of the broad-based terebratellacean type set low on the dorsal mantle, and the filaments when extended in feeding are directed anteriorly and ventrally. The structure of the adult lophophore is described. The ciliary feeding mechanism is essentially similar to that described by the author in *Macandrevia cranium* (Müller) and other brachiopods with plectolophous lophophores. D.A.

BATHAM, E. J., PANTIN, C. F. A. & ROBSON, E. A., 1961. The nerve-net of *Metridium senile*: artifacts and the nerve-net. *Quart. J. micr. Sci.*, Vol. 102, pp. 143–56.

Conflicting statements about the actinian nervous system in earlier literature made it necessary to repeat some of the methods of previous workers, and to compare them with present authors' results after using more specific techniques. Criteria for distinguishing nerve cells from non-nervous elements in actinians are discussed. Various structures which may be confused with nerve cells are described, and the results of several earlier authors are re-interpreted. It is concluded that the nervous system in the mesenteries and column of *Metridium* follows the epithelial layers and does not penetrate the mesogloea. C.F.A.P.

BONE, Q., 1961. The organization of the atrial nervous system of amphioxus (*Branchiostoma lanceolatum* (Pallas)). *Phil. Trans.*, Ser. B, Vol. 243, pp. 241–69.

A detailed histological description is given of the rich, mainly sensory, peripheral atrial nervous system of amphioxus. The system contains large numbers of peripheral sensory neurons, whose function is almost entirely unknown and, in addition, contains motor fibres to the striated pterygial and trapezius muscles, and ciliary-regulator fibres to the gill apparatus. Particular attention is paid to the organization of the system upon the pterygial muscle, upon the gill apparatus and atrio-coelomic funnels, and upon the foregut and its diverticulum; in addition, the innervation of the buccal and hindgut regions (outside the atrium and atrial nervous system, but linked closely to the atrial system) are described. It is concluded that the atrial nervous system of amphioxus differs in its function, in its components, and in its anatomical relations, from the autonomic nervous systems of the craniates. Q.B.

Boycott, B. B., 1961. The functional organization of the brain of the cuttlefish *Sepia officinalis*. *Proc. roy. Soc.* B, Vol. 153, pp. 503–34.

Using electrical stimulation as the main experimental method several new divisions of the cuttlefish brain have been made and their responses described. The pedal ganglion consists of four parts: (1) anterior chromatophore lobes innervating the chromatophores of the arms and front of the head; (2) an anterior pedal lobe innervating the arms and tentacles; (3) a posterior pedal lobe innervating the funnel, collar and posterior head retractors; (4) lateral pedal lobes innervating the eye muscles. The palliovisceral ganglion consists of: (*a*) a magnocellular lobe; (*b*) a central palliovisceral lobe innervating the mantle, funnel and viscera; (*c*) posterior chromatophore lobes innervating chromatophores on the mantle, fin and back of the head; (*d*) vasomotor lobes.

Among the supraoesophageal lobes higher motor centres and silent areas are recognized. Of the higher motor centres the anterior basal lobe is concerned with the positioning of the head, arms and eyes and manœuvring of the animal by means of the fins and funnel. The posterior basal lobe consists of six main divisions of which the lateral and medial basal lobes control chromatophore and skin movements; the medial basal lobes also control swimming, breathing, fin movements and various visceral functions. The interbasal lobe controls tentacle movements.

The optic nerves and cortex of the optic lobes are electrically inexcitable. The medulla of the optic lobes evokes all the responses of which the higher motor centres are capable.

The results are discussed in terms of a physiological classification of the brain and the extent to which centres controlling behaviour can be recognized within a brain.

B.B.B.

Carlisle, D. B., 1961. Locomotory powers of adult ascidians. *Proc. zool. Soc. Lond.*, Vol. 136, pp. 141–6.

Adults of several species of both solitary and colonial ascidians are capable of slow crawling, involving the progressive formation of new attachments and tearing or dissolving the old ones. The ampullae play a leading part. A moving colony may divide into two, or two adjacent colonies may fuse; true fusion has been observed only once, in *Trididemnum tenerum*. Both *Ciona intestinalis* and *Diplosoma listerianum* may metamorphose by attachment to the surface film for a prolonged period and are capable of post-metamorphic attachment to a solid surface. In such attachment the ampullae play a leading role. A free-swimming, unattached colony of *Diplosoma* takes on the form of a *Pyrosoma* colony, and such colonies have been reared in the laboratory, forming cigar-shaped, jet-propelled bodies up to 1 cm long, with as many as fifty zooids.

D.B.C.

Carlisle, D. B., 1961. Intertidal territory in fish. *Anim. Behaviour*, Vol. 9, pp. 106–7.

Bass (*Morone labrax*) and grey mullet (*Mugil labrosus*) were observed on an intertidal reef on the coast of south Devon. The same individual fish were seen to arrive each day as soon as the water was deep enough and to stay until just after high tide. Each fish occupied a definite territory on the reef and defended it against others of its own species. The method of defence is described, together with notes on the feeding habits.

D.B.C.

CARLISLE, D. B. & PITMAN, W. J., 1961. Diapause, neurosecretion and hormones in Copepoda. *Nature, Lond.*, Vol. 190, pp. 827–8.

Neurosecretory cells in the cerebral region of *Calanus* were found to be inactive in overwintering fifth copepodid larvae. It is concluded that the activity of these cells is needed for continued moulting and development, so that this 'diapause' is a result of the cessation of their secretory activity and is terminated by a recrudescence of this activity. A chromactivating substance active upon *Leander* has been extracted from the same region. D.B.C.

HINKE, J. A. M., 1961. The measurement of sodium and potassium activities in the squid axon by means of cation-selective glass micro-electrodes. *J. Physiol.*, Vol. 156, pp. 314–35.

Intracellular measurements were made on isolated axons of *Loligo forbesi* in sea water. At 18° C, $(a_{Na})_i = 0{\cdot}037$, $(a_K)_i = 0{\cdot}203$. The sum of $(a_{Na})_i$ and $(a_K)_i$ was always constant. The calculated equilibrium potential for Na equalled or exceeded the reversal potential observed from the axon spike. The calculated equilibrium potential for K was about 20 mV higher than the observed resting potential. The activity coefficient of axoplasm was calculated as 0·605. When K^+ was assumed to be 100% free, Na^+ was 76% free in the axoplasm. Following electrical stimulation, the net inward and outward fluxes were 3·9 and 5·8 pmoles/cm²/impulse for Na^+ and K^+ respectively

J.A.M.H.

NICHOLS, D., 1961. A comparative histological study of the tube-feet of two regular echinoids. *Quart. J. micr. Sci.*, Vol. 102, pp. 157–80.

The principal tube-feet of regular sea-urchins have terminal suckers supported by a skeleton; sensory cells, mucous glands and muscles are so arranged that there is both an efficient discharge of slime and an effective suction under the disk during adhesion. The common British urchin *Echinus*, which uses its tube-feet for locomotion, is shown to have advanced in respect of these features over the more primitive *Cidaris*, which uses its tube-feet mainly for support. The peristomial tube-feet of both urchins are not suckered; the disk levator muscles are absent and the disk itself, supported by a less complex calcareous skeleton, contains mainly sensory cells and mucous glands. The tube-foot pattern of the more advanced irregular urchins, the clypeasteroids and spatangoids, is shown to be derivable from the basic cidaroid type.

Differences in the ornamentation of the regions of the test which bear the tube-feet are shown to be related to the differences in activity.

A respiratory function for Stewart's organs in the Cidaroida is suggested. D.N.

ROBINSON, A. H. W., 1961. The hydrography of Start Bay and its relationship to beach changes at Hallsands. *Geogr. J.*, Vol. 127, pp. 63–77.

The role of the offshore zone in relation to the changes taking place on a shingle beach is discussed with reference to Start Bay in Devon. The hydrography of the Bay is examined from the standpoint of bottom form, sediments, marine processes and the changes which have occurred in the Skerries Bank during the past century. A record of beach changes at Hallsands on the shores of the Bay indicates a paucity of material

available for beach building. The beach is best regarded as an inheritance from the past, with little or no material arriving on the shore from the adjacent sea bed. The reason for this negative role of the offshore zone is discussed, the available evidence pointing to a movement of sediment parallel to the shore under the action of tidal streams rather than towards the shore in the direction of the dominant wave.

A.H.W.R.

Ross, D. M., 1960. The effects of ions and drugs on neuro-muscular preparations of sea anemones. I. On preparations of the column of *Calliactis* and *Metridium*. *J. exp. Biol.*, Vol. 37, 732–52.

Drugs and ions affect isolated rings of the columns of *Calliactis* and *Metridium* as follows: (1) direct contractions and higher background tonus occur with excess K^+, Mg^{2+}-free sea water, adrenaline (1×10^{-5}) and tryptamine (1×10^{-4}); (2) marked acceleration of slow periodic activity accompanies Mg^{2+}-free sea water and adrenaline (1×10^{-5}); (3) enhancement of slow responses to stimulation occurs with excess $-Ca^{2+}$ and tryptamine (1×10^{-5}), (4) depression of activity and abolition of responses is seen only with excess $-Mg^{2+}$ and Ca^{2+}-free sea water. K^+-free and Na^+-free sea water have slight effects only.

No drugs with depressant effects were discovered. Most other drugs are ineffective (e.g. acetylcholine and its associates, noradrenaline, tyramine; and adrenergic blocking-agents; histamine; and 5-hydroxytryptamine). The observed effects with K^+, adrenaline, and tryptamine are due to a direct action on or near the muscle. Comparisons with other animals and preparations show: (1) the actinian neuromuscular system resembles a number of marine animals in its responses to ions; (2) except for the sensitivity to adrenaline, no parallels exist with drug effects on any other system, so that no suggestions about the mechanism of neuromuscular transmission can be made on the basis of these results.

D.M.R.

Ross, D. M., 1960. The effects of ions and drugs on neuro-muscular preparations of sea anemones. II. On sphincter preparations of *Calliactis* and *Metridium*. *J. exp. Biol.*, Vol. 37, 753–74.

Isolated marginal sphincters of *Calliactis* and *Metridium* show little inherent activity, but with appropriate frequencies and numbers of stimuli, they give quick facilitated contractions and/or slow contractions with long latent periods. Excess K^+ causes spontaneous quick contractions, and responses to single stimuli; excess Ca^{2+} has no direct excitatory effects but causes an immense enhancement of the quick response without affecting the slow response to stimulation; excess Mg^{2+} abolishes both quick and slow contractions. Most drugs, including acetylcholine and its associates, histamine and 5-hydroxytryptamine, have no effects on either quick or slow contractions. No drug with inhibitory properties was discovered.

Important effects occur only with the following: (1) tyramine (1×10^{-4}) has no direct excitatory effects, but it greatly enhances the quick responses without enhancing (and in some cases depressing) the slow responses to stimuli; (2) tryptamine (1×10^{-4}) causes direct quick contractions of the sphincter, gives quick responses to single stimuli and somewhat enhances both quick and slow responses to stimuli; (3) adrenaline (1×10^{-5}) has no effect on quick facilitated responses, but causes direct slow responses and some enhancement of slow responses to stimuli.

The effects of ions and drugs are obtained only when they are applied directly on or near the sphincter region. It is noted that on the column and sphincter preparations K^+ and tryptamine act alike, as do Ca^{2+} and tyramine, whereas adrenaline has a general slow excitor action that has no counterpart amongst the ion effects. The results suggest that the quick and slow contractions of the sphincters share common excitability factors but differ in the mechanism of contraction. The process of facilitation in the quick response is apparently not functionally related to the mode of excitation of the slow response. D.M.R.

SOUTHWARD, E. C., 1961. Pogonophora from South Africa. *Ann. S. Afr. Mus.*, Vol. 46, pp. 47–52.

The first member of the Pogonophora to be described from the south Atlantic is *Heptabrachia talboti* n.sp., collected off the west coast of South Africa in depths between 1097 and 2890 m. Empty tubes of two other, unidentified, pogonophores are also described. E.C.S.

BOOK REVIEWS

Ecology and Distribution of Recent Foraminifera

By Fred B. Phleger

The Johns Hopkins Press, Baltimore, 1960. Pp. viii + 297, 11 plates and 83 text-figures. Price £3.

In this country, studies of the Foraminifera have been confined to a few workers, the group having been on the whole somewhat neglected by marine biologists in recent years. In the United States, on the other hand, considerable advances in our knowledge of this group have been made, due no doubt to impetus from the petroleum industry. Older records seldom take into account whether tests found in bottom sediments were alive or not, so that it is refreshing to find a work dealing with the Foraminifera as living organisms. It is well known that remains of Foraminifera accumulated on the sea floor are of value in tracing the sequence of climatic and other changes in the past, and a study of the ecology and distribution of recent forms, both planktonic and benthic, shows that they may also be useful indicators of the present-day marine environment. Prof. Phleger's book brings together for the first time much of the original work published in scientific journals in recent years, to form a reference work of equal value to the geologist as to the ecologist and oceanographer. N.A.H.

Oysters

By C. M. Yonge

New Naturalist Special Volume, Collins, London, 1960. Pp. xiv + 209, 17 plates and 72 text-figures. Price 21*s*.

From the presence of enormous numbers of oyster shells in prehistoric kitchen middens it would appear that the oyster was one of the more important items in the diet of primitive man. The Romans valued the English oyster most highly, and through the centuries it has been held in the greatest esteem, but it was not until the middle of the last century, consequent on the decline of natural oyster beds, that scientific cultivation of the oyster was begun. This book covers all aspects of the biology of the oyster, with particular reference to the European flat oyster, *Ostrea edulis*. Opening chapters describe the evolutionary origin, structure and reproduction of the oyster, followed by an account of the occurrence of oysters in other parts of the world. Much is now known of the food requirements of oysters, both in the larval and adult stages, and these and other conditions necessary for successful spat-fall and growth are described in detail. The oyster has many enemies and these include a number of predatory gastropods, and the slipper-limpet which, although not a predator, hinders both the growth and harvesting of the oyster by occurring in such numbers as to smother the beds.

The chapter on 'Oysters in the Past' covers not only fossil forms, but man's early exploitation of the oyster for food. Oyster culture from Roman times to the present day, and the means of cultivating and collecting in different parts of the world are described.

Prof. Yonge's attractive and informative book is illustrated by many line drawings and photographs, together with reproductions of some charming engravings and lithographs. N.A.H.

Radioactive Wastes. Their Treatment and Disposal

Edited by J. C. Collins

Foreword by Sir Alexander Fleck, K.B.E., D.Sc., F.R.S.

Published by E. and F. N. Spon Ltd, London. 1960. Pp. xxi + 239, 1 plate, 46 text-figures, 12 tables. Price 55*s*.

This volume encompassing both the principles and current practices of radioactive-waste disposal is written by eight authors, specialists in the various fields concerned. Despite a distributed authorship the book is uniformly written in an easy and simple style well adapted to meet the needs of readers unacquainted with nuclear physics and its jargon. Particularly valuable are the numerical examples and detailed calculations, for this is a field where misunderstandings are intolerable. The worked examples and the many line drawings and graphs undoubtedly help to make the subject-matter fully and readily intelligible. It is a pity that the extent and importance of secondary biological accumulation is dealt with so briefly. This deficiency, together with lack of discharge figures and environmental data, prevent the reader from arriving at his own objective assessment of current practices. In this respect it should have been clearly emphasized that the acceptable contamination of sea water ought not to be determined by drinking-water tolerances, as the example on p. 188 might suggest. Nevertheless, it is to be hoped that this small volume will go some way to dispelling the less informed criticism sometimes levelled at practices in radioactive-waste disposal and help to give a clearer picture of the problems involved.

T. I. S.

Current Bibliography for Aquatic Science and Fisheries

Compiled by FAO Fisheries Biology Branch

Published by Taylor and Francis Ltd, London. Printed by Warren and Son Ltd, The Wykeham Press, Winchester. Price per part 17*s*. 6*d*.: subscription per vol. of 12 pts. £9.

This Bibliography, which is compiled and prepared by the Biology Branch of the FAO Fisheries Division, is now appearing in a new format published by Messrs Taylor and Francis Ltd. The first two volumes were distributed in mimeographed form and the new style running from Vol. 3 (1960) is a great improvement. The publication is a most important bibliographic tool for all organizations or individuals concerned with the fields of marine or freshwater research.

The bibliography consists of annotated references to work published all over the world in serial publications, books, theses, translations, mimeographed reports or films. Each reference is clearly set out and marked to indicate whether its emphasis is marine or freshwater. The scope and coverage is stated as 'in general limited to documents. . .referring to studies of living aquatic resources and their environments, or to methods of research applicable in this field', but clearly this has been interpreted in a very generous way. The first issue of Vol. 3 contains detailed explanatory notes on the general arrangement, annotations etc. and exhaustive indexes will be provided for each volume (Author, Geographic, Taxonomic, Subject and Citation). Geographic, taxonomic and subject classifications and codes are given in detail and demonstrate the wide coverage of the Bibliography. Most helpfully, suggestions are also given for the method of binding of completed volumes.

The editors and all who have helped to prepare the Bibliography are to be congratulated on the result.

L. M. S.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1960–61

The Council have great pleasure in reporting that Prof. C. F. A. Pantin, Sc.D., F.R.S., was elected President of the Association in June in place of Prof. A. V. Hill, C.H., O.B.E., F.R.S., who had served for the previous five years.

The Council wish to record their deep appreciation of the services rendered to the Association by Prof. A. V. Hill during his term of office, a period notable for the increased development of research on the physiology of marine organisms at the Plymouth laboratory. Prof. Hill was re-elected a Vice-President of the Association.

The Council have to report with regret the death of the Earl of Verulam, a Vice-President of the Association since 1953; and of Mr W. H. Searle, B.E.M., who served the Association so faithfully as fisherman collector for a period of 63 years.

The Council and Officers

Four ordinary meetings of the Council were held during the year, two in the rooms of the Royal Society, one in the rooms of the Zoological Society of London and one at Plymouth. At these the average attendance was seventeen.

The Plymouth Laboratory

During the year the outside woodwork and chuting of the south wall of the centre bay of the south building have been repainted.

The small lean-to coal shed on the inner side of the south wall of the yard near the entrance gate has been converted by direct labour to house the motor and compressor for charging aqualung cylinders with air.

As ancillary work to the main laboratory extension, the plunger jar room has been renovated and refurnished for use by the finance office staff.

A new oil-fired furnace has been installed in the basement of the south building and a new flue has been built in place of the existing lift shaft. The chimney pots have been removed from all the flues of the south building.

Laboratory Extension

The Council have signed a contract with Messrs John Garrett and Son (Contractors) Ltd for the building of the new laboratory extension for which

Messrs Easton & Robertson, Cusdin, Preston and Smith, London, are the Association's Architects.

Work on the extension began on 2 May 1960 and the building is well advanced.

Aquarium

A detailed description of the aquarium, reservoirs and outside circulation tanks has been published by Dr Wilson in Vol. 39, No. 3, of the *Journal*.

During the summer months the aquarium has been crowded on most days, often to capacity, and the public have evidently appreciated its general appearance and the animals exhibited. There is no doubt that the new conditions suit the animals well, and the improved servicing facilities have made maintenance much easier.

Two electrically driven Mono Pumps were installed in the basement engine room in February 1961 to replace the two centrifugal pumps now over thirty years old. The new pumps have stainless steel bodies, rubber stators and stainless steel rotors. Each delivers over 4500 gallons an hour, which would appear to be more than twice the amount the old pumps have given for many years. The increased flow of water through the tanks was quickly followed by increased activity of many of their inhabitants, and analyses showed a marked improvement in the oxygen saturation of the water in the tanks. The increased flow is assisting silt removal from the tanks while not interfering with silt deposition in the reservoirs, and the water remains remarkably clean.

Research Ships

The three research vessels R.V. 'Sarsia', R.V. 'Sula' and M.V. 'Gammarus' have operated regularly throughout the year.

In December 1960 R.V. 'Sula' went into dock for the Lloyds' special survey of the hull. Apart from deterioration round the stern the vessel, which is now 12 years old, was found to be in excellent condition. The sternpost, which was of English elm, has been renewed in oak. At the same time the engine was lifted and completely overhauled.

The vessel is now expected to be fit for many years' service, and her classification continues to be A 1 at Lloyds.

Tercentenary of the Royal Society

On Monday, 18 July 1960, in the Rooms of the Royal Society, Burlington House, London, the Secretary, Dr F. S. Russell, F.R.S., presented a congratulatory address (see opposite) from the Council of the Association to the President of the Royal Society on the occasion of the celebration of the tercentenary of the Society's foundation. This address was inscribed by

TO THE PRESIDENT OF THE ROYAL SOCIETY OF LONDON

The Council of the Marine Biological Association of the United Kingdom sends congratulatory greetings to the Royal Society of London on the occasion of its Tercentenary. They recall that the Association was formed in 1884 at a meeting held in the rooms of the Society under the chairmanship of Thomas Henry Huxley, then its President. They are grateful for all the help which the Society has given to the Association in many ways during the intervening years, a period which is already one quarter of the life of the Society itself.

The Royal Society has always had a representative Governor on the Council of the Marine Biological Association: and many members of the Association are, or have been, Fellows of the Society. Very distinguished contributions to marine science have been made by Fellows who have been on the staff of the Plymouth Laboratory: and a host of Fellows of the Society have worked at Plymouth.

In recent years there has been a great development of interest, on an international scale, in the sciences of the sea. The Royal Society, by long tradition, has always had a special interest in international cooperation, and today several of its National Committees are concerned with different aspects of research in marine science. Such work is of fundamental interest to the Association, and its members find it a matter of the greatest satisfaction that our national contribution to it is being guided by the Royal Society.

F. H. Pantin

18 July 1960. President.

(*Facing p.* 838)

Mr A. Forrest, A.R.C.A., A.T.D., Principal of the Plymouth College of Art, and contained in a tooled blue leather scroll case.

Staff

The Honorary Degree of Doctor of Science has been conferred on Dr F. S. Russell, F.R.S., by the University of Exeter.

Mr Q. Bone has been promoted to the grade of Senior Scientific Officer.

Dr D. P. Wilson attended the Congrès International d'Aquariologie at Monaco from 21–26 November and also visited the Marine Laboratory at Villefranche.

Dr L. H. N. Cooper, as Chairman of the Committee on Chemical Oceanography, attended the meeting of the International Association of Physical Oceanography in Helsinki from 25 July to 6 August.

Dr Mary Parke took part in a joint excursion of the British Phycological Society, of which she is President, and the Société Phycologique de France in the Channel Isles in September.

Dr E. J. Denton and Dr T. I. Shaw spent a month during April and May at the marine laboratory at Villefranche studying the buoyancy of plankton animals.

Dr D. B. Carlisle attended the First International Congress of Endocrinology in Copenhagen in July and spent much of that month working at the Kristinebergs Zoologiska Station in Sweden.

Mr N. A. Holme attended a special meeting held under the auspices of the International Council for the Exploration of the Sea at Heligoland in June to review methods of quantitative research on the fauna of the sea-floor.

Mr F. A. J. Armstrong spent a month in May and June on board R.R.S. 'Discovery II' taking part in the International Council for the Exploration of the Sea combined expeditions to investigate the outflow of cold deep northern water over the Iceland–Faroe Ridge. From 25 July to 6 August he attended the meeting of the International Association of Physical Oceanography in Helsinki.

Occupation of Tables

The following one hundred and thirty-eight workers have occupied tables at the Plymouth Laboratory during the year.

Dr A. Acara, Istanbul (Physical Oceanography).
E. Adams, Plymouth (Library).
Dr W. J. Adelman, Bethesda, U.S.A. (Squid axons).
Dr J. S. Alexandrowicz, Plymouth (Nervous system of invertebrates).
D. C. Arnold, Durham (Library).
Dr Daphne Atkins, Plymouth (Brachiopods).
Dr J. R. Baker, F.R.S., Oxford (Cytology of dogfish pancreas).
P. F. Baker, Cambridge (Biochemistry of squid axons).
W. J. Ballantine, D.S.I.R., London (Ecology of limpets).

Dr ANNA BIDDER, Cambridge (*Heligocranchia pfefferi*).
Dr E. J. BINYON, London (Ion exchange in *Asterias rubens*).
Dr G. T. BOALCH, International Paints Research Fellow (Effects of toxic substances on algae).
G. C. BOLSTER, Lowestoft (Mackerel).
Dr A. D. BONEY, Plymouth (Ecology of red algae).
Mrs P. C. BORJA, Philippines (General).
Prof. A. A. BOYDEN, New Jersey (Comparation serology).
Dr ELEANOR M. BROWN, London (Plankton).
Dr P. C. CALDWELL, Alan Johnston, Lawrence and Mosely Research Fellow of the Royal Society (Muscle and nerve physiology).
Mrs P. A. CALDWELL, Plymouth (Library).
R. CALVERT, Surbiton (Testing equipment at sea).
N. L. CHADWICK, Salisbury (Library).
D. M. CHAPMAN, Cambridge (Scyphozoan hydrotuba).
W. CHAPPLE, Syracuse, U.S.A. (Library).
Miss E. CLAY, Brixham (Library).
A. J. COLLINGS, Birmingham (Volatile organic matter in sea water).
J. S. COLMAN, Port Erin (Deep-sea plankton).
R. H. COOK, Cambridge (Library).
Miss A. C. COUPLAND, Birmingham (Library).
Dr J. W. COWIE, Bristol (Submarine geology).
E. CROWTHER, Southampton (Library).
N. CUMMINS, Plymouth (Library).
D. CURRY, Bristol (Submarine geology).
R. J. DANIEL, Torpoint (Library).
J. M. DIAMOND, Cambridge (Sodium ions in bile).
P. S. B. DIGBY, London (Pressure sensitivity in marine animals).
Dr ELIZABETH J. DIMELOW, New Brunswick (Biology of *Antedon*).
Sister MARIE THERESE DIMOND, Washington (Radio-iodine uptake in fish embryos).
L. A. W. DOWNER, Tavistock (Library).
M. EDMUNDS, Oxford (Defence mechanisms in nudibranchs).
Dr MARIA M. FELINSKA, Ashby de la Zouch (Ciliates).
A. E. FISHER, N.I.O., Wormley (Algal culture techniques).
K. A. FLETCHER, Surbiton (Testing equipment at sea).
I. H. FORD, Bristol (Submarine geology).
R. FOWLER, Plymouth (Library).
C. J. FOXWELL, Plymouth (Library).
Dr VERA FRETTER, Reading (Prosobranch molluscs).
Dr KAREN R. GAARDER, Oslo (Systematics of flagellates).
Dr J. B. GILPIN-BROWN, Plymouth (Biology of *Nereis fucata*).
B. GLAIZNER, London (General).
Dr D. R. GLASSON, Plymouth (Library).
J. GOSTAN, Villefranche (Chemical oceanography).
Prof. A. GRAHAM, Reading (Prosobranch molluscs).
Mrs G. GRANT, Chalfont St Peter (*Mytilus* veligers).
J. R. GRINDLEY, Capetown (Library).
E. HARADA, Kyoto (Crustacean larvae).
Dr D. N. HARCOURT, Plymouth (Library).
M. G. HARDY, Reading (Euphausiacea).
Dr H. W. HARVEY, F.R.S., Plymouth (Productivity of sea water).

J. J. Hatch, Ashburton (Library).
J. B. Hawthorne, Weymouth (Library).
D. Heddle, Oxford (Functional morphology of asteroids).
Dr R. B. Hill, Glasgow (Innervation of *Buccinum* heart).
Dr D. Hines, Plymouth (Library).
Dr J. A. Hinke, London (Squid axons).
Prof. A. L. Hodgkin, F.R.S., Foulerton Research Professor of the Royal Society (Squid axons).
Dr E. P. Hodgkin, Perth, W. Australia (Littoral biology).
D. J. Hume, Teignmouth (Library).
O. D. Hunt, Newton Ferrers (Library).
Miss A. Hurst, Reading (*Philine*).
Mrs W. Hussain, London (Algal culture techniques).
Dr W. G. Inglis, London (Free-living nematodes).
Dr C. H. Jellard, Plymouth (Library).
Dr F. R. H. Jones, Lowestoft (Labyrinth fluids of elasmobranchs).
P. G. W. Jones, Lowestoft (Chemistry of sea water).
G. C. Kearn, Birmingham (Monogenean parasites of fish).
Sister Mary St John Keefe, Boston (Food storage in *Turritella*).
Dr G. Y. Kennedy, Sheffield (Chlorophyll pigments).
Dr R. D. Keynes, F.R.S., Cambridge (Physiology of squid giant axons).
Prof. L. H. Kleinholz, Oregon (Crustacean endocrinology).
Prof. E. W. Knight-Jones, Swansea (Library).
Dr Joan Lance, Southampton (Behaviour of *Calanus*).
Dr Marie V. Lebour, Plymouth (Decapod larvae).
Miss R. S. Lee, Oxford (Nervous system of tunicates and echinoderms).
D. Leston, London (Biology of *Aepophilus*).
J. G. E. Lewis, Bradford (Littoral centipedes).
Dr J. Llewellyn, Birmingham (Trematode parasites of fishes).
Prof. O. E. Lowenstein, F.R.S., Birmingham (Central nervous responses in elasmobranchs).
R. McGregor, Parkstone (Library).
Prof. H. McLennan, Vancouver (Physico-chemical properties of nerve tissue extracts).
G. I. Mann, Plymouth (Library).
Miss J. E. Manning, London (Hydroids).
Prof. Irene Manton, F.R.S., Leeds (Flagellates).
Dr A. L. Martin, London (Library).
Dr J. J. Milaire, Brussels (Cytochemistry of dogfish embryos).
J. Moorey, N.I.O., Wormley (Measurement of temperature and salinity with portable salinometer).
Miss M. A. Murphy, Manchester (Maritime Apterygota and dogfish skin).
Dr J. S. C. Parry, Bristol (Submarine geology).
Dr R. B. Pike, Millport (Decapod larvae).
W. J. Pitman, Ohio (Endocrinology).
Dr W. T. W. Potts, Birmingham (Body fluids of elasmobranchs).
Cdr. C. F. B. Powell, R.N. (Rtd.), Plymouth (Library).
Dr J. N. Prebble, London (Library).
Mrs J. N. Prebble, London (Library).
M. Quick, Bristol (Submarine geology).
Dr W. J. Rees, London (*Stygiomedusa*).
Dr J. M. Ritchie, New York (Heat production in muscle and nerves).

Dr M. B. V. Roberts, Marlborough (Annelid histology).
Dr Elaine Robson, Cambridge (Nervous system of *Calliactis* and *Metridium*).
H. Rosa, jr., Rome (Library).
Dr D. M. Ross, London (*Calliactis* and *Eupagurus*).
Miss H. G. Q. Rowett, Plymouth (Library).
A. S. M. Saleuddin, Reading (*Astarte*).
P. D. V. Savage, Plymouth (Library).
D. J. Scarratt, D.S.I.R. (Fauna of *Laminaria* holdfasts).
A. Sharples, Plymouth (Library).
Miss J. Shephard, London (Polyzoa).
Dr A. J. Smith, Bristol (Submarine geology).
Prof. J. E. Smith, F.R.S., Ray Lankester Investigator, London (Nervous system of *Echinus*).
Dr Eve C. Southward, Plymouth (Pogonophora; polychaetes).
B. W. P. Sparrow, Newton Ferrers (Library).
Miss F. A. Stanbury, Plymouth (*Cladophora*).
Prof. M. Tamashige, Sapparo (Crustacean stretch-receptor).
Dr W. H. Thomas, La Jolla (General).
C. H. Thorp, Newton Ferrers (Library).
Dr K. K. Tiwari, Calcutta (Crustacean endocrinology and systematics).
D. L. Urry, London (*Pseudocalanus*).
R. C. Vernon, London (Library).
Sister Anna Voss, California (Plankton).
Dr R. A. Wall, Oxford (Peptides of *Rhodymenia*).
H. Wallace, Belfast (Ascidian embryology).
P. R. Walne, Conway (Library).
G. E. Walster, Plymouth (Glycolysis in *Maia*).
Prof. W. F. Whittard, F.R.S., Bristol (Submarine geology).
F. W. Williams, Zanzibar (Pelagic fish).
Dr D. I. Williamson, Port Erin (Decapod larvae).
P. Woodhead, Lowestoft (Elasmobranch thyroids).
Mrs A. D. Woodhead, Lowestoft (Elasmobranch thyroids).
Mrs J. A. M. Wootton, London (Biosynthesis of sterols).

Among the many other scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Mrs C. Wallace (New Zealand), W. D. Oliff (South Africa), G. Boillot (France), Dr J. B. Bateman (U.S.A.), Dr G. L. Clarke (U.S.A.), Mrs C. L. Deeleman (Holland), K. Boss (U.S.A.), Dr T. S. Austin (U.S.A.), Dr M. C. Sargent (U.S.A.), Dr B. Komarovsky (Israel), Dr F. J. Schwarz (U.S.A.), B. Schiefflin (U.S.A.), Prof. J. L. Mohr (U.S.A.), Dr B. C. Abbot (U.S.A.), Prof. S. O. Hörstadius (Sweden), Dr A. Krishnakumarau (India), N. H. Verdonk (Holland), A. O. Simpson (South Africa), Miss A. Berner (Norway), Prof. R. F. Nunnemacher (U.S.A.), Dr G. Camougis (U.S.A.), Prof. M. L. Fott (Czechoslovakia), Dr A. H. Leim (Canada), M. Bashuruddin (Pakistan), Dr V. R. Meenakshi (India), Dr J. C. van de Kamer (Holland), Prof. D. L. Ray (U.S.A.), Dr J. S. Craigie (Canada), Dr W. H. Thomas (U.S.A.),

F. Körte (Germany), Dr M. Neushul (U.S.A.), Dr Eugenie Clarke (U.S.A.), J. E. King (U.S.A.), Prof. C. Kloom (Thailand), Dr M. Yamada (Japan), Dr E. S. Herald (U.S.A.), J. M. Thiemmedh (Thailand), J. L. Pepys-Cockerell (Solomon Islands), Prof. F. J. Trembley (U.S.A.), Prof. and Mrs J. B. Lackey (U.S.A.), Dr G. A. Botros (Egypt).

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin and were attended by forty-one students from the following Universities: Oxford, Cambridge, Reading, Exeter, Aberystwyth, Southampton, London, Sheffield, Glasgow, Leicester, Edinburgh, and Hull.

Also during the Easter Vacation Dr R. F. Jones and Dr C. T. Prime brought a party of nine boys from Whitgift School and four boys from other schools and Mrs R. M. Goldsmith brought a party of five girls from Harrow County School.

Scientific Work of the Plymouth Laboratory Staff

Sea Water and Plankton

Dr L. H. N. Cooper has continued his revision of the hydrographic observations made in the Celtic Sea in 1950. He has had help from Cdr. A. L. Lawford, R.N. (Rtd.) and Instructor-Cdr. V. F. C. Veley, R.N. in re-examining the current measurements which they had made at the Seven Stones Lightvessel in collaboration with Dr J. N. Carruthers. Conflicting evidence has been harmonized in terms of a narrow, intermittent, well-mixed corner current around Land's End from south to north. The mean direction of the current at the lightvessel had been established as towards 110°. The re-examination has shown that the current tends to deviate to the south of the mean direction at neap tides and in summer and to the north of the mean direction at spring tides and in winter. All the observations now fall into place. The water north of Cornwall tends therefore to be recruited during neap tides in a narrow current around Land's End. The biological and meteorological consequences north of Cornwall of the corner current have been examined.

Much of the Celtic Sea is dominated by wind drift currents. However, the prevailing winds from west and south-west tend to produce a 'prevailing' wind-drift towards south-east and south. Land topography constrains the direction of the wind-drift near coasts where more persistent current regimes may be found. The cyclonic eddy, long recognized, seems to be confined to the north-eastern Celtic Sea. The biological consequences have been examined. Much of this work has been published in Vol. 39, Nos. 2 and 3, of the *Journal*.

In May 1960, earlier evidence for stratification of the deep Atlantic based on oxygen samples obtained from faulty water-bottles was confirmed by samples believed to be free from suspicion. Observations obtained in 1956 suggest that massive upward displacements of all the water between 300 and

4000 m depth may occur. It amounted to 70–200 m. At the meeting of the International Association of Physical Oceanography in Helsinki, Dr Cooper presented a historical account of the overspill over the northern ridges of cold, heavy Arctic water into the North Atlantic. At a symposium there on comparative chemical oceanography, he described the influence that these ridges exert on world chemical oceanography.

Mr F. A. J. Armstrong and Mr E. I. Butler have continued the monthly cruises to the International Hydrographic Station E 1 and have reported the analyses for 1959 in Vol. 39, No. 3, of the *Journal*. Their surveys, started in 1959, of an area of 30 by 45 miles, off Plymouth, which includes the Station E 1, have been repeated bimonthly in 1960. The patterns of water properties have varied considerably and appear to show that Atlantic water may enter the area from the south-east, south, south-west and even north-west. In May an attempt was made to measure water movement at E 1 with cone-type current meters. It failed because of lack of sensitivity in the meters, but direct measurement is still needed to avoid ambiguity in interpreting movement from the hydrographic observations. During the complete tidal period occupied by the current measurements a series of bathythermograph readings were also taken. It showed an irregular oscillation of the thermocline with an amplitude of about 5 m. The record was too short to determine the periodicity.

Mr Armstrong and Dr G. T. Boalch have continued their measurements of the ultra-violet absorption spectra of samples of sea water, and it seems clear that, at least down to a wavelength of 250 mμ, the spectra are continuations of those measured at longer wavelengths by Kalle and others, and that high absorption is associated with the yellowish matter found in coastal water. At shorter wavelengths other constituents may increase the absorption, and it is likely that very high values found in deep North Atlantic water are caused by nitrate ion. The work on ultra-violet absorption and chemistry of distillates from sea water and plant cultures has continued. The seasonal variation of absorption in the 220–250 mμ range was repeated in 1960, maximum values having been found in September. A fairly constant amount of volatile sulphur-containing material has been found throughout the year. For the estimation of carbon in the volatile material a simple combustion method has been developed. The quantities found (which may be a small part of the total because of losses which cannot yet be avoided) have varied from 30–300 μg C/l. and so represent some 5% or so of the dissolved organic carbon in the water. Identification of these volatile constituents is difficult because even in the distillates the concentrations are probably less than 10 mg/l. However, it is hoped that eventually a gas chromatograph may be assembled for this work. Some tests with volatile matter for biological activity in plant cultures and with *Echinus* larvae gave inconclusive results. An account of Mr Armstrong's earlier work on suspended matter appeared in Vol. 17 of the *Journal*

of Marine Research, and he has written a short article on sea-water fertility for the new McGraw-Hill Encyclopaedia of Science and Technology.

Dr T. I. Shaw has continued experimental work upon the state of iodine in sea water. The exchange of ^{131}I between oxidized and reduced forms of iodine in sea water appears to be only of occasional occurrence. A titrimetric apparatus has been prepared for further studies.

During the year Dr Mary Parke and Miss Irene Adams have prepared two papers for the press. The first, for publication in the *Bulletin of the Research Council of Israel*, dealt with a problematic green flagellate, most interesting from the phylogenetic viewpoint. The second paper on the motile (*Crystallolithus hyalinus*) and non-motile phases in the life-history of the coccolithophorid, *Coccolithus pelagicus*, has been published in Vol. 39, No. 2, of the *Journal*. In the same number of the *Journal* the results of the study on the 1–2μ green flagellates, in connexion with the naming of '*Chromulina*' *pusilla* Butcher, have been published by Dr Mary Parke in co-operation with Prof. Irene Manton.

Valuable data are still being accumulated by Dr Parke and Miss Adams on the life-histories of a number of organisms in the Plymouth collection. Although still in its early stages, this work has already shown that a number of the marine algal forms that occur on the shore and have been described as benthic Chrysophyceae are in reality non-motile stages in the life-histories of some inshore coccolithophorids. It is hoped also in this way to elucidate the life-history and find also the correct systematic position for the puzzling planktonic organism *Halosphaera viridis* Schmitz which has now been obtained in culture. Other interesting flagellate forms have also been isolated by Miss Adams, of particular interest being the freshwater *Chrysochromulina* from Lake Windermere which is almost certainly the type species of the genus. Dr Parke is co-operating with Prof. Irene Manton and Dr J. W. G. Lund in the study of this organism.

Dr D. P. Wilson and Mr F. A. J. Armstrong have again turned their attention to problems raised by their previous work on biological differences between sea waters from different localities. There have recently been suggestions by continental workers that the differences they found were due to differing copper concentrations in the waters used. It has been shown by these workers that when copper is added to sea water in which sea-urchin (*Paracentrotus lividus*) larvae are reared the larvae are adversely affected by the copper, even at such relatively low concentrations as are occasionally to be found in natural sea waters. It seemed imperative to test these suggestions with *Echinus esculentus* larvae, using sea water from at least two contrasted localities, as in previous experiments at Plymouth, and not sea water from only one locality as in the continental work just mentioned. Accordingly a number of tests have been carried out with water from E1 and from the Clyde. It was found that these two waters each gave a distinctly different morpho-

logical type of larva, which was clearly recognizable. This was so up to a copper concentration higher than that ever likely to be found in the localities from which sea waters contrasted in earlier experiments had been obtained. It was found that the addition of copper did indeed at higher levels of concentration stunt the growth of the larvae, but even at the highest levels tested the morphological type peculiar to each natural water was recognizable. Water from one source always gave better and different larvae than water from the other source. Moreover, the removal, by chelation, of ionic copper from the two waters did not destroy this clear biological difference although it did result in somewhat poorer larvae in both waters so chelated. Indeed a slight addition of copper to the better of the two waters gave markedly improved larvae without affecting their morphological type. Mixtures of the two waters produced interesting results and in one or two comparisons better larvae were obtained in mixed water than in either alone. Some other tests were also made. The data obtained are now being studied. Larvae from some of the experiments were preserved; these have been mounted and are being examined microscopically for features not so readily comparable while the larvae were alive.

Dr E. D. S. Corner has completed a preliminary investigation of the nutrition of the copepod *Calanus helgolandicus*. Adult females were kept for short periods in a continuous flow of 'outside' Plymouth sea water, and measurements were made of the quantity and type of food they assimilated during the months April–September. It was found that *Calanus* preferentially selected a diet of high organic content from the particulate food available, and the total quantity assimilated was equivalent to a respiration rate which adequately accounted for the highest values reported by previous workers using *C. finmarchicus*. This result provides direct evidence that, during summer months at least, *Calanus* obtains the bulk of its food from insoluble substances present in the sea, dissolved material making no substantial contribution to its diet. An account of this work has been published in Vol. 41, No. 1, of the *Journal*. It is hoped to continue the study during the ensuing year by determining the respiration rate of *C. helgolandicus* under conditions identical with those used in the present feeding experiments.

Dr F. S. Russell, in collaboration with Dr W. J. Rees of the British Museum (Nat. Hist.), has published a detailed account of the viviparous scyphomedusa, *Stygiomedusa fabulosa*, in Vol. 39, No. 2, of the *Journal*. This medusa has an unusual method of asexual reproduction. The young medusae are developed in capsules which project into the cavities of four brood chambers. These capsules result from cysts which develop along a germinal line in the endoderm of the brood pouch and have tubular outgrowths into the stomach cavity. The cyst acts as a chorion passing on nourishment to the medusa which develops within it.

Dr Russell has also examined a collection of deep-water medusae made by R.R.S. 'Discovery II' for the vertical distribution investigations of Dr Talbot

H. Waterman of Yale University. Among these he has found a new trachymedusan of the genus *Colobonema*, a description of which appeared in Vol. 41, No. 1, of the *Journal*.

Dr A. J. Southward has completed the first of two papers on the distribution of zooplankton indicator species in the English Channel and Celtic Sea. This paper, published in Vol. 41, No. 1, of the *Journal*, deals with samples taken with conventional nets in 1955 and 1957; the second, in preparation, describes the results of high-speed sampling in 1958–60. The evidence suggests that changes in distribution of zooplankton since the 1900's, and associated changes in the fisheries, may be related to the general rise in temperature that has taken place during the period. An approximate treatment of some temperature data has been published in Vol. 39, No. 3, of the *Journal*.

Macro-fauna and Flora

The results of Dr A. J. Southward's researches during the past five years on the cirral activity of sessile barnacles are embodied in a paper in association with Dr D. J. Crisp. From cinematographic records, supplemented by direct observation where possible, five different types of activity have been recognized, and their relationship to feeding and respiratory exchange investigated: they are, in order of increasing muscular activity: testing, pumping, normal beat, fast beat, and extension. Pumping appears to be mainly respiratory, microfeeding can occur during pumping and normal beat, while captorial feeding on large organisms takes place during normal beat, fast beat and extension. Fast beat and extension appear to be particularly adapted to captorial feeding, the former in still water, the latter in moving water.

Study has now turned to duration of cirral activity, especially inherent rhythms, in relation to environmental changes, and much time has been spent devising electronic recording gear. This investigation has been delayed by the resumption of building operations at Plymouth, since the animals are very sensitive to mechanical shock. However, excellent results were obtained during a visit to the Marine Biology Station (University College of North Wales) at Menai Bridge. Tidal rhythms seem to be lacking, but shorter period rhythms present in both intertidal and sublittoral species appear to be sensitive to water movement and to oxygen content and hydrogen ion content of the water.

Many herbarium specimens have been added to the Plymouth collection of marine algae this year, in particular material from the Channel Islands collected by Dr Parke, Miss Adams and Miss Hearn during the Phycological field meeting there in September. Amongst the recent additions are specimens of species either occurring rarely or not previously recorded for the British Isles.

Mr G. M. Spooner has continued studies of the interstitial fauna of marine

gravels, particularly with reference to the malacostracan Crustacea. A description of the new *Ingolfiella* has been published in Vol. 39, No. 2, of the *Journal* and that of two new species of bogidiellids (a family of small hypogean species near Gammaridae) is being prepared. These forms (together possibly with an unknown elongate species of the tanaid genus *Strongylura*) appear, as far as the evidence goes, to be distributed sparsely through the body of the gravel and not concentrated close to the surface, as are the greater bulk of malacostracans isolated from the gravel samples. This is in keeping with the striking distribution of known species of Ingolfiellidae and Bogidiellidae, suggesting that these families belong to a special fauna that has developed in and dispersed from the subsurface water held in the pores or joints of the earth's strata. This hypogean fauna must be supposed to spread in the water held in the deposits of the ocean floor as well as in subterranean ground water contained below land surfaces.

For comparison with the Eddystone Rock, a collection of encrusting algae was made from almost the most northerly point of the Isles of Scilly, at low water. The fauna proved rich, including larvae of the little-known marine midge *Psammathiomyia pectinata*, and two isopods new to Great Britain, *Jaeropsis brevicornis* Koehler and *Munna petti* Amar. The latter is known otherwise only from the Mediterranean. *Jaeropsis brevicornis* was originally described from Sark over 70 years ago and has since been recorded in small numbers from a few localities southward to Italy. Though ten other species are now recognized from other parts of the world, very little is known about the genus.

Mr P. G. Corbin has continued to collect data on the Lucernarians, hermit crabs and some of the smaller fishes. At Portwrinkle, in September 1960, Mr R. J. Daniel found a single specimen of the *Lucernariopsis* sp. which hitherto has only been taken plentifully at Wembury. A lot of material has been examined from other localities but so far the species has been found very sparsely outside its Wembury site; at Portwrinkle (1), Looe (1) and Scilly (2). There would appear to be nothing exceptional about the Wembury habitat. In this respect, it parallels the exceptionally restricted distribution of the Connemara Sucker, *Lepadogaster candolli*, at one site near Brixham. The small hermit crab, *Anapagurus chiroacanthus*, has been found to occur in small numbers offshore. It is not recorded in the Fauna List, but is not new to the Plymouth area as Mr D. J. Macdonald previously found it when working on pagurid larvae in 1933 and 1934. Macdonald's manuscript records of the species were received at the laboratory only recently.

Mr N. A. Holme has completed a report on the bottom fauna of the English Channel, based on a series of anchor-dredge hauls along the south coast of England. An additional cruise was made in December 1959 to fill in a gap in the records between Plymouth and Dartmouth, and in all 173 stations have now been worked. A brief outline of trends in distribution

was given in the last *Report*, and additional work has confirmed the previous observations. Temperature appears to be an important factor in the distribution of species near the northern end of their range, but the restriction of certain other forms either to the western end or to the western half of the Channel appears to be related to other factors. While high turbidity in the eastern parts of the Channel may be contributory, no single factor has emerged to explain these distributions, and it seems possible that it is due to the presence of some unknown factor in certain waters.

In February 1960 the dredge survey was extended, a number of transects across the eastern Channel and a series of stations along the Normandy coast being worked. There seems to be some evidence of a greater up-Channel penetration of certain species in mid-Channel and on the French side, but the results have yet to be worked up in detail.

While on holiday in May 1960 Mr Holme visited the Marine Laboratories at Concarneau and Roscoff and toured an oyster farm in the Gulf of Morbihan.

Measurements were made by Mr G. R. Forster of the sample of prawns referred to in last year's report, but the results obtained did not agree with the correlation previously found between the length of the O-group and water temperatures. After a particularly warm summer the length of the prawn was no greater than in some of the previous colder years. Unfortunately, the place from which these samples have been taken on this occasion was heavily fished by some professional prawners. It is felt that growth rate problems could best be resolved by maintaining an experimental population of small prawns in a large tank for a few months.

In July Mr Forster made a short cruise in R.V. 'Sarsia' to try out a deep-sea long line which he has designed. Although the weather curtailed operations the line was shot six times at depths of 500–1000 fm. in the vicinity of La Chapelle bank. A total of thirteen fish were taken. These included three species of deep-water dogfish, *Centroscymnus coelolepis*, *Etmopterus princeps* and *Centrophorus squamosus*; two black scabbard fish *Aphanopus carbo* and five deep-sea eels *Synaphobranchus* sp. were also taken. These results are similar to those obtained by Prince Albert of Monaco who undertook a considerable amount of deep-sea long-line fishing over 50 years ago. It is hoped that by spreading the hooks farther apart larger catches may be obtained. Very little appears to be known both about the abundance of fish and their food on the deeper parts of the continental slope where it is not generally possible to trawl.

Mr Forster has also continued diving near Plymouth though bad weather has frequently interrupted this work. A short survey was made during August on the Lulworth Bank near Weymouth with the co-operation of the marine survey section from the Winfrith Heath Atomic Energy Research Station. In the survey quantitative estimates were made of the abundance of three species: *Polymastia boletiformis* (sponge), *Bispira volutacornis* (polychaete

worm), and *Lepralia foliacea* (bryozoan). A total area of over 600 m^2 was covered. The results were published in Vol. 41, No. 1, of the *Journal*.

Mr Q. Bone has continued the study of the nervous system of amphioxus: a detailed account of the organization of the central nervous system has been published in the *Journal of Comparative Neurology*, and an account of the innervation of the skin has been published in Vol. 101 of the *Quarterly Journal of Microscopical Science*. The former provides the first identification of the viscero- and somatic-motor cells within the cord, and shows that the system in adult amphioxus is not unlike that found in the early stages of lower craniates. The latter describes a special type of sense-organ from the skin of the ventral region of the body, and considers the implications of the arrangement of the innervation of the skin of the animal with regard to recent theories of the mechanism of skin sensibility. An account of the atrial nervous system of the animal has appeared in the *Philosophical Transactions of the Royal Society*.

Some hauls with a new bottom sampling net designed to collect larvae of amphioxus were carried out late in the season, in collaboration with Mr Forster; it is hoped to use this gear next season and obtain material for a study of the ecology of the larval stages.

The central and peripheral nervous systems of cyclostomes are being studied at present, especial attention being paid to *Myxine*, specimens of which were obtained in Sweden by Dr D. B. Carlisle.

The above researches on primitive chordates are being made as a necessary preliminary leading on to investigations on the organization of the spinal cord in fishes in relation to their swimming behaviour.

Physiology of Marine Organisms

A histological study of the subocular light-organs of stomiatoid fishes, started last year by Dr J. A. C. Nicol, has now been completed, and an account of this work has been published in Vol. 39, No. 3, of the *Journal*. These organs are directly innervated, and it seems that light-production is subject to nervous control. In addition, some method of occluding the light-organ is always present. In the majority of stomiatoids there is a special muscle, arising from the hyomandibular bone, that passes underneath the light-organ to an insertion on the lower and outer face of the latter. Contraction of this muscle pulls the light-organ downwards and hides it from view. In *Idiacanthus* the same muscle is present, but it passes up in front of the light-organ and is inserted on a sheet of black tissue above. Contraction of this muscle pulls a screen down over the light-organ of *Idiacanthus* and screens it from the exterior. This study clarifies certain aspects of the physiology of luminescence in marine fishes.

Dr Nicol has also prepared an account of animal luminescence for the first volume of the *Advances in Comparative Physiology and Biochemistry*.

Continuing investigations begun in 1955, Dr Nicol has been making a comparative and functional study of the tapetum of elasmobranch fishes. In these animals there is a layer of shining platelets in the chorioid, which throw light back upon the retina and cause the fundus of the eye to appear highly reflective. This arrangement confers increased sensitivity on the eye. It is generally believed that the elasmobranch tapetum is occlusible, i.e. that it is covered over by processes of black pigment cells in bright light, and is exposed in darkness. An extensive and exhaustive study has demonstrated that the tapetum is fixed in the dogfish (*Scyliorhinus*) and the rays. In these animals there is always a black patch in the ventral fields, where the eye receives light from above, and a bright retinal surface elsewhere. The tapetum is occlusible in the spur dog (*Squalus acanthias*). Studies are in progress to elucidate the mechanism of pigment-movement in the tapetum of *Squalus* and to determine how it is induced and controlled. This investigation is being extended to other species of elasmobranchs as they become available.

Dr E. J. Denton, in collaboration with Dr J. B. Gilpin-Brown, has continued to study the buoyancy of the cuttlefish. *Sepia officinalis* (L.) is usually active during the night and buries itself during the day. The change of behaviour with light intensity is associated with marked changes in the animal's buoyancy; in the light it becomes less buoyant, in the dark more buoyant. These changes in density, which may amount to 2%, are given by changes in the density of the cuttlebone. After being kept for 2 days in complete darkness a cuttlefish usually becomes so buoyant that it is incapable of staying at the bottom of its tank and can only remain in mid-water with difficulty. The cuttlefish uses its cuttlebone in the same way that a submarine commander uses the buoyancy tanks of his craft; for example, to become less dense the cuttlefish pumps water out of the cuttlebone and increases its gas space. A short account of this work has been published in the *Journal of Physiology*, Vol. 151.

With Mr J. V. Howarth, Dr Denton and Dr Gilpin-Brown have shown that in the first 6 h after cuttlefish are hauled up from about 40 fathoms to the surface of the sea the equivalent osmotic concentration of the liquid inside the cuttlebone moves from about 75% to about 97% of sea water. A small extrapolation back to the time at which hauling began gives a figure for the osmotic concentration in good agreement with their osmotic hypothesis, namely, that when the cuttlefish is at depth in the sea it holds liquid out of the cuttlebone by matching an osmotic difference between cuttlebone liquid and blood against the external hydrostatic pressure. Whilst the concentration of salts in the cuttlebone liquid is rising the liquid deeper inside the cuttlebone is hypotonic to that close to its outer wall. They have explained this quantitatively in terms of the slowness of diffusion along the narrow chambers of the cuttlebone. These chambers are inclined at small angles to the siphuncular surface of the bone so that to reach a depth of about 2 mm from the surface of

the bone the salts have to diffuse along about 8 mm of chamber. The slowness of change of composition is clearly advantageous to the cuttlefish, for it allows transient changes of depth to disturb the salt concentration of the liquid inside the bone very little. The buoyancy of the cuttlefish has been shown to be very insensitive to changes in pressure at least up to six atmospheres. In this it is in marked contrast to a fish with a gas-filled swim-bladder.

They have shown that to go about half way towards an equilibrium between carbon dioxide in the gas space of the cuttlebone and carbon dioxide in the surrounding tissues would take about 1 day. For a given partial pressure difference nitrogen, the gas principally found in the cuttlebone, only diffuses at 1/40th the rate of carbon dioxide and to reach half equilibrium would therefore take about 40 days. This explains why the mass of gas within the cuttlebone remains constant when the cuttlebone density changes.

Dr Denton and Dr T. I. Shaw have studied, in the marine laboratory at Villefranche, the buoyancy of some of the many gelatinous planktonic animals. They found that all the animals studied, including ctenophores, medusae, heteropods and pteropods, were close to neutral buoyancy. The stroma, or structural components of these animals, is always denser than sea water and has a specific gravity of 1·3–1·4, characteristic of protein. Buoyancy is given by the body fluids which are less dense than sea water and supply an upthrust of the order of ½mg/ml. The water soluble material forms over 99·8% of the total body weight.

Some animals (particularly medusae and ctenophores) are less dense than sea water. This is considered to be an arrangement whereby the animals will be kept near the surface of the sea. Even if these animals were entirely inactive, turbulence in the water will tend to hold them below the surface—this has been clearly seen with an experiment using a model animal. When turbulence increases in bad weather the animals will automatically go deeper without effort on their own part.

Whilst at Villefranche Dr Denton and Dr Shaw took the opportunity of visiting Monaco where they examined the visual pigment of the moray eel. They found it to be intermediate in colour between the retinal pigments of the ordinary coastal fish and of the conger eel. The moray eel thus differs from the conger, the silver freshwater and the deep-sea eels, all of which have been shown to have the golden pigment characteristic of deep-sea fish.

Dr Denton gave a communication on 'The design of fish and cephalopod eyes in relation to their environment' in a Symposium at the Zoological Society. An account of this communication is being published in the *Proceedings of the Zoological Society*.

Dr E. D. S. Corner, Dr E. J. Denton and Mr G. R. Forster have studied the buoyancy of the deep-sea shark *Centroscymnus coelolepis*. This animal was found to be slightly less dense than the sea water at the surface of the sea. The low density is entirely attributable to the very large amount of fat stored in

the liver. The liver accounted for about 28% of the animal's weight, and contained about 80% (by volume) fat; without its liver the fish would have been about 4·5% denser than sea water. By allowing for the differences in compressibility and temperature coefficient of expansion between the tissues and sea water, it was shown that the fish would have been almost perfectly neutrally buoyant at the bottom of the sea from which they came. The low density 0·88 of the fat in the liver of this fish is achieved by storing two-thirds of the fat as the hydrocarbon squalene, which with a density of 0·86 is 60% more efficient at giving buoyancy than normal fat with its density of 0·92.

The investigation into the sexual endocrinology of protandric hermaphrodite decapod Crustacea has been continued by Dr D. B. Carlisle, with particular reference to the effects of the vas deferens gland on female characters. He has found as a result of his visit to Kristinebergs Zoologiska Station that there is in fact no such effect in *Pandalus* or *Calocaris*, so that it is possible for the male and female characters to exist simultaneously in the same individual, and an animal in which the testis has disappeared may still possess the secondary male characters. In testing his working hypothesis about the control of the sexual characters in *Pandalus* he has succeeded by appropriate extirpation and injection in bringing about a reversal in the external characters from female to male, i.e. in the direction opposite to that which normally occurs, and in producing animals which have the appendages of both sexes. He found no effect upon the secondary or primary sexual characters of *Palaemon*, when purified sheep follicle-stimulating hormone was injected in a variety of doses. A paper by Dr Carlisle read to a symposium of the Society for Endocrinology on sexual differentiation in Crustacea has been published in the *Memoirs of the Society for Endocrinology*, No. 7, pp. 9–16.

In collaboration with Mr W. J. P. Smyly of the Freshwater Biological Association, and Mr W. J. Pitman a visiting worker from Ohio State University, Dr Carlisle has begun investigations into the endocrinology of copepods. One of the colour change hormones familiar from the study of decapods has been demonstrated in several species, something of the histology of the endocrine system has been revealed and changes in secretory activity correlated with the occurrence of the diapause in overwintering fifth copepodid stages. A preliminary note on some of this work has been submitted for publication in *Nature*.

A gift of a few milligrams of derivatives of the newly synthesized substance 5,6-dihydroxtryptamine from Dr H. Schlossberger of the Max-Planck-Institut, Munich, has enabled him, with assistance from Dr K. K. Tiwari, a visiting worker from the Zoological Survey of India, to confirm that one of the heart-beat hormones of crabs is in fact this compound.

Dr Carlisle has observed that the brief spell of seemingly uncontrolled cell-division that takes place in the epidermis of a prawn shortly before the moult bears superficial resemblances to the similar uncontrolled cell-division

of vertebrate epithelium during carcinogenesis. Preliminary experiments with a series of injections of well-known carcinogenic compounds have proved promising and suggest that this likeness may be more than superficial. Interest centres upon the means by which the prawn may bring this spate of cell-division under control again.

In collaboration with Dr P. E. Ellis of the Anti-locust Research Centre of the Colonial Office, Dr Carlisle has continued the investigation into the comparative endocrinology of moulting in crustaceans and insects. Most of the results which have emerged so far are perhaps more pertinent to insect endocrinology than to crustacean, but it is becoming apparent how similar is the control in the two groups, and that most of the seeming differences which have been advanced by earlier workers in the field derive from the different ways of thinking about hormones which have arisen in the two fields—the differences seem to be largely semantic. In connexion with this work he has developed a method of softening chitin for histology which has been the subject of a recent publication in *Nature*, Vol. 187. He also delivered a paper at the second symposium of the Zoological Society of London on moulting cycles and this has now been published in *Symposium of the Zoological Society of London*, No. 2, pp. 109–20.

Dr E. D. S. Corner, in collaboration with Dr A. D. Boney of Plymouth Technical College, has continued a study of factors affecting the ecological distribution of intertidal red algae. It has been found that when sporelings of *Plumaria elegans* are screened with dilute solutions of the fluorescent dye Eosin Yellow their growth is markedly increased over a wide range of light intensities, including some below and some above the optimum value of 315 lux. An identical result is obtained when the dye in the screening solution is replaced by phycoerythrin extracted from the adult plant. Further experiments have shown that the stimulation of growth is caused neither by shading nor by exclusion of ultra-violet light, but by protection from blue-green light inhibiting growth. It seems from this that the theory of complementary chromatic adaptation does not apply in the case of *Plumaria*, and further studies along similar lines are planned with other red algae. A preliminary account of this work was published in *Nature*, Vol. 188, p. 1042.

Dr Boney and Dr Corner, in collaboration with Dr W. Carruthers of the Carcinogenic Substances Research Unit, University of Exeter, have been studying the effects of certain carcinogens on the growth of red algal sporelings, and initial experiments have shown that growth is greatly increased by such substances as 9:10-dimethyldibenzanthracene and 20-methylcholanthrene. Further work, in collaboration with Dr G. Y. Kennedy of the Cancer Research Unit, University of Sheffield, has been concerned with the effects of various carcinogens on the phospholipide contents of red algae.

Mr G. R. Forster has continued to assist Dr G. Y. Kennedy in experiments on the regeneration in the sponge *Microciona atrasanguinea*. It was found

that a dilute solution of caffeine markedly hindered the initial reaggregation of dissociated cells. The work is now being carried on by Dr Kennedy at the Cancer Research Unit of Sheffield University with sponge material collected at Plymouth generally by diving.

Dr T. I. Shaw has continued investigations relating to the active transport of ions by the giant nerve fibres of *Loligo*. An account of the effects of injecting various phosphate esters upon the active transport of sodium and potassium by these nerve fibres has been published in collaboration with Prof. A. L. Hodgkin, F.R.S., and Dr R. D. Keynes, F.R.S., of Cambridge, and Dr P. C. Caldwell, Alan Johnston, Lawrence and Mosely Research Fellow of the Royal Society. This account, together with another concerning the partial inhibition of active transport, jointly published with the same co-workers, have appeared in the *Journal of Physiology*, Vol. 152. Further work with the same collaborators has been directed towards determining the fate of phosphate esters which affect sodium transport when they are injected into poisoned axons. It has been found that injections of arginine phosphate very rapidly bring about the regeneration of adenosine triphosphate to a level close to that found in unpoisoned axons. Injections of phospho-enol pyruvate bring about regeneration of both adenosine triphosphate and arginine phosphate in poisoned axons. Currently the fate of phosphate esters in poisoned, extruded axoplasms is being investigated in collaboration with Mr P. F. Baker of Cambridge and it has been found that in extruded axoplasm exposed to cyanide the adenosine triphosphate and arginine phosphate are broken down, the rate of inorganic phosphate formation not being very greatly different from that in intact axons in sea water containing cyanide. Both adenosine triphosphate and arginine phosphate persist in unpoisoned extruded axoplasm. Very recently a technique has been devised whereby a giant axon can be perfused with artificial axoplasm; provided the artificial axoplasm has a certain ionic composition the axon will give action potentials.

Dr Shaw is continuing his investigations on the accumulation of iodine by seaweeds. During the year he has published a second paper on respiration and iodide uptake in Vol. 152 of the *Proceedings of the Royal Society*, B.

Mr J. V. Howarth has continued his investigations into the heat production of the anterior byssus retractor muscle of *Mytilus*. The work has been extended to include a study of the thermoelastic properties of the muscle in the 'fused' state. These prove to be analogous to those of striated muscle when the latter is in the 'active' state. This suggests that the molecular architecture is similar in the two states. In the *Mytilus* muscle an actual fall of temperature is observed when the tension increases due to a small, controlled stretch. Such a thermoelastic cooling is an important term in the computation of the energy balance sheet of a muscle, but previously it lacked direct experimental basis and had to be inferred from indirect evidence.

During the summer months Mr Howarth began a study of the heat

production of the limb nerves of *Maia squinado* in collaboration with Dr J. M. Ritchie of the Albert Einstein College of Medicine, New York. They studied the effect of substituting foreign ions for the sodium of the bathing solutions. The substitution of lithium for sodium made no difference to the heat due to a single stimulus at 0° C, but the net heat due to a series of stimuli at higher temperature was diminished. When sodium is replaced by barium both the action potential and the heat production are abolished, but the presence of a trace of sodium results in a greatly enhanced action potential and increased heat production.

Dr G. W. Bryan has continued his work on the accumulation of radioactive caesium by marine invertebrates in relation to potassium balance. In the decapod crustacea studies with ^{134}Cs have been continued in *Carcinus maenas* and extended to *Portunus puber* and *P. depurator*. Work with ^{137}Cs in *Palaemon serratus* was also continued during a 3-week visit to the United Kingdom Atomic Energy Authority Radiobiology Group at Windscale. Experiments with ^{42}K in the above species have shown that K is taken up far more rapidly than Cs (e.g. ten times more quickly in *Carcinus*) and that most of the K in these animals is readily exchangeable. Animals taking up Cs most slowly are those in which ^{42}K uptake is slowest. In marine decapods Cs concentration factors attained at equilibrium by whole animals vary between about 5 and 20 depending on the species and this is increased in diluted sea water. For comparison, experiments with the freshwater crayfish *Astacus fluviatilis* were carried out at Windscale in sea-water dilutions as low as 0·01 %. Under such conditions whole animal concentration factors of more than 100 are reached in about 300 h although the actual attainment of equilibrium is a far slower process than in the marine species.

In order to study the uptake and loss of ^{137}Cs by brackish-water animals the isopod *Sphaeroma hookeri* and the gastropod *Potamopyrgus* (= *Hydrobia*) *jenkinsi* were used. Sodium and potassium balance has been examined in both animals, with the aid of ^{22}Na and ^{42}K, and this related to the rate and extent of ^{137}Cs accumulation. *Sphaeroma* is able to live almost indefinitely in a range of sea-water concentrations between 2·5 and 100 %. Although Cs is taken up more slowly than K the equilibrium concentration factors attained are almost double those for K at all concentrations. Uptake and loss of ^{137}Cs was studied in *Potamopyrgus* in sea-water concentrations varying between 0·1 and 100 % In the more concentrated solutions ^{137}Cs concentration factors at equilibrium are higher than those for K, but in concentrations as low as 0·1 % the factor for K is about 1000 and that for ^{137}Cs about 500 (including shell).

Additional marine invertebrates have been examined under starved conditions. The accumulation of ^{137}Cs and in some cases of ^{42}K has been studied in the anemones *Actinia equina* and *Calliactis parasitica*, the polychaete *Nereis diversicolor*, the gastropods *Littorina littorea*, *Monodonta lineata*, *Ocenebra erinacea*, *Nucella lapillus*, and in the lamellibranch *Mytilus edulis*.

Excluding the weight of the mollusc shells equilibrium concentration factors for ^{137}Cs in these animals are of the order of 10. Concentration factors in tissues are usually rather higher than those for K but appear to be related to them. Cs is not accumulated by any whole animal or organ to a degree which might indicate that it is a necessary element controlled by specific mechanism.

So far all studies have been carried out in unfed animals. Experiments are now in progress on the effects of feeding on ^{134}Cs accumulation in *Carcinus*, and work on other species in collaboration with the Radiobiology Group, Windscale is anticipated.

Dr G. T. Boalch, International Paints Research Fellow, has continued his investigations on the toxic effects of heavy metals on fouling algae. Using bacteria-free cultures of *Ectocarpus* and *Enteromorpha*, the toxic effects of additions of copper and mercury salts to the culture media have been investigated under controlled conditions. The two weeds show marked differences in resistance to these poisons, *Ectocarpus* having a more effective protection against copper whereas *Enteromorpha* is more resistant to mercury. These differences may well be due to differences in the protection afforded by the extracellular products produced by the algae. These extracellular products and their role in reducing toxicity are still under investigation. A few experiments, using copper and mercury salts, have been carried out with a bacteria-free culture of *Phaeodactylum*.

During the past year monthly visits have been made to the River Yealm and a seasonal examination made of the fouling weeds growing on the test rafts of International Paints Biological Research Laboratory, Newton Ferrers. The identification of fouling weeds, often collected in very early stages of development, is sometimes rather troublesome and when in addition the algae are stunted and distorted by poisons certain identification is often impossible.

Library

The thanks of the Association are once more due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

Published Memoirs

Vol. 39, No. 2, of the *Journal* was published in June, and Vol. 39, No. 3, in October 1960.

The following papers, the outcome of work done at the Plymouth laboratory, have been published elsewhere than in the *Journal* of the Association:

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MEMBERSHIP OF THE ASSOCIATION

The total number of members on 31 March 1961 was 1182, being 86 more than on 31 March 1960; of these the number of life members was 140 and of annual members 1042. The number of associate members is four.

GRANT FROM ROCKEFELLER FOUNDATION

The thanks of the Council are due to the Trustees of the Rockefeller Foundation for a generous grant of 30,000 dollars to be made available during the 2-year period beginning 1 April 1960 for the purchase of certain selected pieces of apparatus for physiological and biochemical research.

GRANT FROM WELLCOME TRUST

The thanks of the Council are also due to the Trustees of the Wellcome Trust for a generous grant of £5,000 towards the cost of fittings in the chemical and physiological laboratories and constant temperature rooms, in the new laboratory extension.

GRANT FOR AQUARIUM RECONSTRUCTION

The Council wish to record their grateful thanks for the following donation towards the cost of reconstructing the Aquarium: Granada T.V. £105.

FINANCE

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Private Income. The Council gratefully acknowledge the following generous grants for the year 1960–61:

Fishmongers' Company (£425), The Royal Society (£100), British Association (£50), Physiological Society (£100), The Cornwall Sea Fisheries Committee (£10), The Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s.*), Leeds (£25), Durham (£10. 10*s.*), Manchester (£10. 10*s.*), Sheffield (£10. 10*s.*), Southampton (£15. 15*s.*), Reading (£10. 10*s.*), Nottingham (£10. 10*s.*), Hull (£10. 10*s.*), Exeter (£10. 10*s.*), Leicester (£10. 10*s.*), Gonville and Caius College, Cambridge (£5), and The Zoological Society of London (£10. 10*s.*).

PRESIDENT, VICE-PRESIDENTS, OFFICERS AND COUNCIL:

The following is the list of those proposed by the Council for election for the year 1961–62:

President

Prof. C. F. A. PANTIN, Sc.D., Hon. Dr. Univ., F.R.S.

Vice-Presidents

THE EARL OF IVEAGH, K.G., C.B., C.M.G.
Sir NICHOLAS E. WATERHOUSE, K.B.E.
Col. Sir EDWARD T. PEEL, K.B.E., D.S.O., M.C.
Vice-Admiral Sir JOHN A. EDGELL, K.B.E., C.B., F.R.S.
Prof. A. V. HILL, C.H., O.B.E., Sc.D., LL.D., F.R.S.
Sir EDWARD J. SALISBURY, Kt., C.B.E., D.Sc., F.R.S.
A. T. A. DOBSON, C.B., C.V.O., C.B.E.
Major E. G. CHRISTIE-MILLER
MORLEY H. NEALE, C.B.E.
Prof. Sir JAMES GRAY, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.
G. M. GRAHAM, C.M.G., O.B.E.

COUNCIL

To retire in 1962

J. N. CARRUTHERS, D.Sc., F.Inst.P.
Prof. A. L. HODGKIN, F.R.S.
Prof. O. E. LOWENSTEIN, D.Sc., F.R.S.
Prof. G. E. NEWELL, T.D., D.Sc.
Prof. W. F. WHITTARD, D.Sc., F.R.S.

To retire in 1963

G. E. R. DEACON, C.B.E., D.Sc., F.R.S.
F. C. FRASER, D.Sc.
M. N. HILL, Ph.D.
O. D. HUNT, F.R.S.E.
Prof. G. P. WELLS, Sc.D., F.R.S.

To retire in 1964

Prof. A. V. HILL, C.H., O.B.E., Sc.D., LL.D., F.R.S.
J. W. G. LUND, Ph.D., D.Sc.
C. H. MORTIMER, Dr.Phil., D.Sc., F.R.S.
Prof. J. T. RANDALL, D.Sc., F.R.S.
H. G. VEVERS, M.B.E., D.Phil.

Hon. Treasurer

HARRISON S. EDWARDS, Westhumble Lacey, near Dorking, Surrey

Secretary

F. S. RUSSELL, C.B.E., D.S.C., D.F.C., LL.D., F.R.S.
The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

B. C. ENGHOLM (Ministry of Agriculture, Fisheries and Food)
The Worshipful Company of Fishmongers:
The Prime Warden
Major E. G. CHRISTIE-MILLER
HARRISON S. EDWARDS
Prof. Sir ALISTER HARDY, Kt., D.Sc., F.R.S. (Oxford University)
S. SMITH, Ph.D. (Cambridge University)
EDWARD HINDLE, Sc.D., F.R.S. (British Association)
N. B. MARSHALL (Zoological Society)
Prof. Sir JAMES GRAY, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)

BALANCE SHEET 1960–61

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET

31 March 1961

	£	£
Capital Reserve Account:		
As at 31 March 1960	167,821	
Add: Expenditure on fixed assets recovered	4,779	
	172,600	
Less: Transfer to surplus account being an amount equivalent to the depreciation provided on assets acquired out of Development Fund grants	3,674	
		168,926
Surplus Account:		
As at 31 March 1960	12,261	
Add: Transfer from Capital Reserve Account	3,674	
Stock adjustment in respect of prior year	1,455	
	17,390	
Deduct:		
Increase in provision for diminution in value of General Fund investments 100		
Excess of expenditure over income for the year ... 5,891		
	5,991	
		11,399
		180,325
Balances on Special Funds (see annexed statement)		4,574
Current Liabilities:		
Sundry creditors and accrued expenses	6,045	
Subscriptions and grant received in advance	363	
		6,408
		£191,307

Note: Capital commitments outstanding amount to approximately £74,500 (1960 £2,730) of which £73,900 (1960 £1,500) is recoverable

J. E. Harris } *Members of the Council*
O. D. Hunt }

	£ Cost or Valuation	£ Depreciation	£
Fixed Assets:			
Boats and equipment:			
At cost:			
R.V. 'Sarsia'	137,761	13,077	124,684
M.F.V. 'Sula'	12,500	1,605	10,895
R.L. 'Gammarus'	200	40	160
	150,461	14,722	135,739
Laboratory apparatus, equipment and machinery: At cost	22,868	8,186	14,682
Library at valuation in 1941 plus additions as valued by the Director	25,290	—	25,290
	£198,619	£22,908	
			175,711
Investments at Market Value:			
General Fund (including Composition Fees) at book amount (Market value £1,472; last year £1,368)		2,019	
E. T. Browne Bequest Funds at cost (Market value £3,264; last year £3,400)		4,915	
		6,934	
Less: Provision for diminution in value of investments		2,198	
			4,736
Current Assets:			
Stocks on hand at the lower of cost and estimated realizable value ...		5,776	
Sundry debtors and prepayments		2,628	
Balances at bankers and cash in hand		2,456	
			10,860
			£191,307

Auditors' Report to the Members of the Marine Biological Association of the United Kingdom:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31 March 1961 and of its excess of expenditure over income for the year ended on that date.

We have obtained all the information and explanations which we considered necessary. In our opinion the Association has kept proper books and the said accounts which are in agreement with them and with the said information and explanations, give in the prescribed manner the information required by the Companies Act 1948.

Norwich Union House
2 St Andrew's Cross
Plymouth
19 May 1961

Price Waterhouse & Co.
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT

	£	£
Salaries (including £6,722 for previous years) National Insurance, Superannuation Scheme Contributions and Supplementary Pensions ...		54,993
Laboratory's and Boats' Crews' Wages (including £308 for previous years), National Insurance, Superannuation Scheme Contributions, Pensions and Employers' Liability Insurance ...		38,147
Upkeep of Library ...		858
Scientific Publications, *less* Sales ...		1,849
Upkeep of Laboratories:		
Buildings and machinery ...	595	
Electricity, gas, coal and water ...	1,335	
Chemicals and apparatus ...	2,835	
Depreciation of laboratory apparatus, equipment and machinery ...	1,631	
Rates (including £1,038 for previous year) ...	2,221	
Rents and insurance ...	431	
Travelling expenses ...	1,013	
Audit fee ...	179	
Stationery, postage, telephone and sundries ...	1,739	
Specimens ...	96	
Collecting expenses and upkeep of truck ...	328	
		12,403
Maintenance and Operation of Boats:		
Petrol, oil, paraffin, etc. ...	1,811	
Maintenance and repairs ...	10,641	
Depreciation ...	3,674	
Insurances ...	2,413	
Hire of Decca Navigator—R.V. 'Sarsia' ...	395	
		18,934
Entertainment Expenses ...		63
		£127,247

FOR THE YEAR ENDED 31 MARCH 1961

	£	£
Grants and Table Rents:		
Ministry of Agriculture, Fisheries and Food—Grant from Development Fund (including £7,030 for previous years) ...	111,332	
Fishmongers' Company ...	400	
Miscellaneous (including Royal Society £100, British Association £50, Physiological Society £100, Universities of London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s.*, Leeds £25, Southampton £15. 15*s.*, Durham £10. 10*s.*, Exeter £10. 10*s.*, Leicester £10. 10*s.*, Manchester £10. 10*s.*, Nottingham £10*s.* 10*s.*, Hull £10. 10*s.*, Reading £10. 10*s.*, and Sheffield £10. 10*s.*, Zoological Society of London £10. 10*s.*, Ministry of Works £104, Imperial Chemical Industries Ltd., £52. 10*s.*, International Paints Ltd., £52. 10*s.*, Gonville and Caius College, Cambridge £5) ...	1,533	
		113,265
Subscriptions ...		1,156
Sales:		
Specimens ...	3,426	
Fish ...	546	
	£	
Nets, gear and hydrographical equipment ...	1,597	
Less: Cost of materials ...	1,179	
	418	
		4,390
Interest on Bank Deposits, less charges ...		56
Income from Investments ...		64
Aquarium:		
Admission fees ...	3,235	
Sale of guides ...	104	
	3,339	
Less: Maintenance, printing and advertising ...	914	
		2,425
Balance being excess of expenditure over income for the year ...		5,891
		£127,247

MOVEMENTS ON SPECIAL FUNDS DURING THE YEAR TO 31 MARCH 1961

	E. T. Browne Bequest									
	Library £	Special Apparatus £	Scientific Publications £	Library Reserve Fund £	Rockefeller Foundation Fund £	Aquarium Reconstruction Fund £	Reservoir and Sea Water Tanks Fund £	Main Laboratory Extension Fund £	Research Funds* £	TOTAL £
BALANCES AT 31 MARCH 1960 (after providing £1,417 for diminution in value of investments)	1,059	1,936	753	232	—	1,240	(193)	—	338	5,365
Add: Income during year										
Grants	—	—	—	—	1,587	—	193	36,280	3,839	41,899
Amounts paid direct to contractors and suppliers by:										
Nuffield Foundation	—	—	—	—	—	1,500	—	—	—	1,500
Rockefeller Foundation	—	—	—	—	3,961	—	—	—	—	3,961
Income from investments	40	84	36	—	—	—	—	—	—	160
Bank deposit interest	—	—	—	—	—	43	—	—	—	43
Other income	—	—	69	—	—	132	—	—	—	201
	1,099	2,020	858	232	5,548	2,915	—	36,280	4,177	53,129
Deduct: Expenditure during year	61	—	—	—	5,878	2,118	—	36,280	3,983	48,320
Increase in provision for diminution in value of investments	60	125	50	—	—	—	—	—	—	235
BALANCES AT 31 MARCH 1961	£978	£1895	£808	£232	£(330)	£797	—	—	£194	£4,574

* Including International Paints Limited Research Fellowship.

LIBRARY
BUREAU OF COMMERCIAL FISHERIES
U. S. FISH AND WILDLIFE SERVICE
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